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REVIEW ARTICLE

Convergence of 3D bioprinting, bone organoids, and organ-on-chip systems for orthopedic disease modeling and regenerative applications

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Abstract

Bone disorders remain difficult to model because native bone is a mineralized, vascularized, mechanosensitive, and multicellular organ whose remodeling depends on tightly coordinated matrix, transport, biochemical, and mechanical cues. This review examines how three-dimensional (3D) bioprinting-enabled bone organoids and organ-on-chip systems can be integrated to construct more physiologically relevant platforms for orthopedic research. Unlike previous reviews that discuss bone organoids, 3D bioprinting, or bone-on-chip systems as separate technologies, this review addresses the conceptual and translational gap between biological self-organization, programmable biofabrication, and dynamic microphysiological regulation in orthopedic modeling. We synthesize current advances in bone microenvironment modeling, focusing on organoid-based biomimicry, programmable scaffold fabrication, perfusable and mechanically active chip systems, and disease-oriented applications. The analysis shows that 3D bioprinting transforms bone microenvironment reconstruction from empirical scaffold fabrication into a controllable strategy, enabling the systematic engineering of matrix composition, pore architecture, mineralization, vascular-like channels, multicellular organization, and biochemical patterning. Organ-on-chip platforms further enhance these constructs by introducing dynamic perfusion, mechanical stimulation, compartmentalized crosstalk, and disease-relevant microenvironmental regulation. Together, these technologies support emerging applications in bone defect repair, osteoporosis and metabolic bone disease modeling, osteoarthritis and osteochondral interface reconstruction, and bone tumor or metastasis research. Despite persistent limitations in tissue maturation, vascular hierarchy, immune and hematopoietic integration, long-term stability, and standardization, the convergence of organoids, 3D bioprinting, and microphysiological systems provides a versatile foundation for mechanistic studies, drug evaluation, personalized therapeutic testing, and future regenerative strategies in orthopedics.

Keywords: 3D bioprinting; Bone organoids; Organ-on-a-chip; Bone microenvironment; Orthopedic disease modeling

1. Introduction

Bone disorders and injuries, including critical-sized defects, osteoporosis, osteoarthritis, and primary or metastatic bone tumors, continue to pose major clinical challenges.[1-3] One central reason is that bone is a mineralized, vascularized, and mechanosensitive organ whose development, remodeling, and pathology are governed by the coordinated regulation of extracellular matrix (ECM) dynamics, multicellular interactions, mass transport, and mechanical cues.[4-6] Although conventional two-dimensional (2D) cultures and animal models have provided important insights, they remain insufficient to fully capture human-specific responses and the integrated complexity of the bone microenvironment.[7,8]

Organoids provide a promising biological foundation for advanced orthopedic models because they preserve three-dimensional (3D) cell–cell and cell–matrix interactions and support tissue-like organization more effectively than monolayer cultures.[9,10] However, spontaneous self-organization alone rarely recapitulates stable mineralized matrix formation, vascular-associated transport, or mechanically regulated maturation in bone.[11,12] In this context, 3D bioprinting offers a practical bridge toward greater structural and functional integration. By enabling the spatially defined assembly of cells, matrices, and bioactive cues, it transforms mineralization-permissive matrices, pore architecture, vascular channels, and multicellular interfaces into programmable design parameters.[13,14] Organ-on-chip systems further provide controlled perfusion, biochemical gradients, and dynamic stimulation; however, chip-only models often rely on simplified tissue architectures and limited cellular complexity.[15,16] These complementary limitations have therefore driven growing interest in integrating organoid-level biological complexity with chip-based microenvironmental regulation.[17,18]

To avoid terminological ambiguity, we use these related terms with distinct meanings in this review. A "bone organoid" refers to a self-organizing or guided self-organizing 3D bone-like tissue model that contains osteogenic-lineage cells and recapitulates selected features of the bone microenvironment, such as mineralized matrix formation, multicellular crosstalk, vascular-associated transport, or remodeling activity. A "spheroid" refers more narrowly to a 3D cell aggregate and does not necessarily reproduce tissue-level organization or function. A "3D-bioprinted construct" or "engineered bone-like tissue" emphasizes externally programmed scaffold architecture, material composition, spatial cell distribution, and biochemical patterning. A "bone-on-chip" system refers to a microphysiological platform that introduces perfusion, compartmentalization, gradients, or mechanical stimulation to model dynamic bone-related processes. An "organoid-on-chip" system integrates organoid-level biological complexity with chip-based microenvironmental regulation. In this review, the term "3D-bioprinted bone organoid-on-chip" is used only when biological self-organization, programmable biofabrication, and dynamic chip-based regulation are combined within the same platform.

Emerging evidence indicates that the convergence of organoid technology, 3D bioprinting, and organ-on-chip platforms constitutes a powerful strategy for constructing advanced *in vitro* bone models.[18-20] By combining the intrinsic self-organizing capacity of organoids with the architectural precision and reproducibility enabled by bioprinting, while incorporating the dynamic and physiologically relevant microenvironment provided by chip-based systems, this integrated approach creates new opportunities to more faithfully recapitulate the structural, cellular, and functional complexity of native bone tissue.[21-23] In doing so, it becomes possible to couple organoid self-organization with reproducible structural control and chip-compatible functionality.[18,20]

Despite rapid progress, the field still lacks a review that critically integrates bone organoids, 3D bioprinting, and organ-on-chip systems from the perspective of bone microenvironment reconstruction. Existing discussions often emphasize one component of the platform, such as organoid self-organization, printable scaffold fabrication, or microfluidic regulation, but they rarely explain how these technologies can be combined to reproduce the defining requirements of bone, including mineralized matrix formation, vascular-associated transport, mechanical regulation, multicellular remodeling, and disease-specific microenvironmental remodeling.

Accordingly, this review is organized around three interrelated issues. First, it defines the biological requirements that bone organoids must satisfy to approximate the native bone microenvironment, including mineralized matrix formation, multicellular remodeling, vascular-associated transport, and mechanical regulation.

Second, it examines how 3D bioprinting converts these biological requirements into programmable material, architectural, cellular, biochemical, and mechanical parameters. Third, it discusses how organ-on-chip systems provide dynamic perfusion, mechanical stimulation, compartmentalized crosstalk, and disease-relevant regulation for orthopedic disease modeling, drug evaluation, and translational application.

The novelty of this review is therefore to frame 3D bioprinting-enabled bone organoids and organ-on-chip systems as an integrated engineering–biological strategy rather than as independent technologies. We propose the central hypothesis that bone organoids provide biological complexity, 3D bioprinting provides spatial and material programmability, and organ-on-chip systems provide dynamic microenvironmental regulation. Their convergence may therefore generate more physiologically relevant, reproducible, and clinically informative platforms for orthopedic disease modeling, drug evaluation, personalized therapeutic testing, and future regenerative applications.

Based on this framework, this review summarizes the essential biological requirements of the bone microenvironment, then discusses how organoid systems approximate these requirements, how 3D bioprinting converts them into programmable material and architectural parameters, and how organ-on-chip platforms introduce perfusion, mechanical stimulation, and compartmentalized crosstalk. Finally, we critically evaluate disease-specific applications, current validation standards, translational barriers, and future priorities for the adoption of orthopedic organoid-on-chip technology.

A narrative literature search was performed in PubMed, Web of Science, Scopus, and Google Scholar up to May 2026 using combinations of the following terms: "bone organoid," "bone-on-chip," "organoid-on-chip," "3D bioprinting," "bioink," "vascularized bone," "osteoporosis," "osteoarthritis," "bone defect," "osteosarcoma," "bone metastasis," "osteomyelitis," "mechanical loading," and "microphysiological system." Studies were prioritized if they reported human-relevant bone/cartilage organoids, 3D-bioprinted bone-like constructs, microfluidic bone models, validation readouts, or orthopedic disease applications. Exclusion criteria included articles focused solely on non-orthopedic soft-tissue organoids, studies that used conventional scaffold fabrication without biological validation, non-peer-reviewed material without sufficient methodological detail, or duplicate records. Because this article is a narrative and conceptual review rather than a meta-analysis, the included studies were synthesized thematically according to biological requirements, engineering parameters, validation criteria, and disease-specific applications.

2. Bone microenvironment

Bone is not only a mechanical scaffold supporting the vertebrate body but also a dynamic endocrine organ that integrates metabolic regulation, hematopoietic support, and mechanosensory functions.[4] Its exceptional performance emerges from the interplay between a highly ordered hierarchical architecture and a complex multicellular ecosystem. At the cellular level, osteoblasts, osteoclasts, and osteocytes interact with endothelial cells, mesenchymal stromal cells, and hematopoietic cells, forming a coupled network that is continuously shaped by the ECM and vascular compartment to maintain microenvironmental homeostasis.

At the material level, bone is a composite built upon type I collagen fibrils and lamellae reinforced by carbonate-substituted hydroxyapatite nanocrystals.[5,24] This precisely coupled organic–inorganic architecture underlies the simultaneous toughness and stiffness required for physiological loading. Importantly, bone is a quintessential mechanosensitive tissue in which biophysical cues are not passive consequences of structure but active regulators of tissue maintenance and lineage commitment.[25] Mechanical inputs can be sensed by osteocyte-associated mechanosensitive structures and transduced into biochemical signaling, thereby coordinating osteoblast-mediated formation and osteoclast-mediated resorption to preserve remodeling balance.[26]

In parallel, the bone microenvironment is enriched with biochemical signals—including growth factors and chemokines—that regulate cellular behaviors and tissue remodeling through dynamic spatiotemporal gradients.[27] These signals coordinate progenitor proliferation and differentiation, couple osteogenesis with angiogenesis, and orchestrate the regulatory programs underlying bone development and repair. In orthopedic disease contexts, this tightly regulated homeostasis is frequently reprogrammed by pathological perturbations.[28] Conditions such as osteoporosis, bone metastasis, and fracture are accompanied by pronounced microenvironmental remodeling, often manifested as disrupted metabolic equilibrium and dysregulated signaling pathways that reshape both cellular crosstalk and matrix turnover.

From a modeling standpoint, the bone microenvironment can be distilled into three recurrent requirements: (i) mineralized matrix and mechanics, (ii) multicellular remodeling programs, and (iii) dynamic transport and mechanoregulation. This framework links bone biology to engineering design by treating spatial

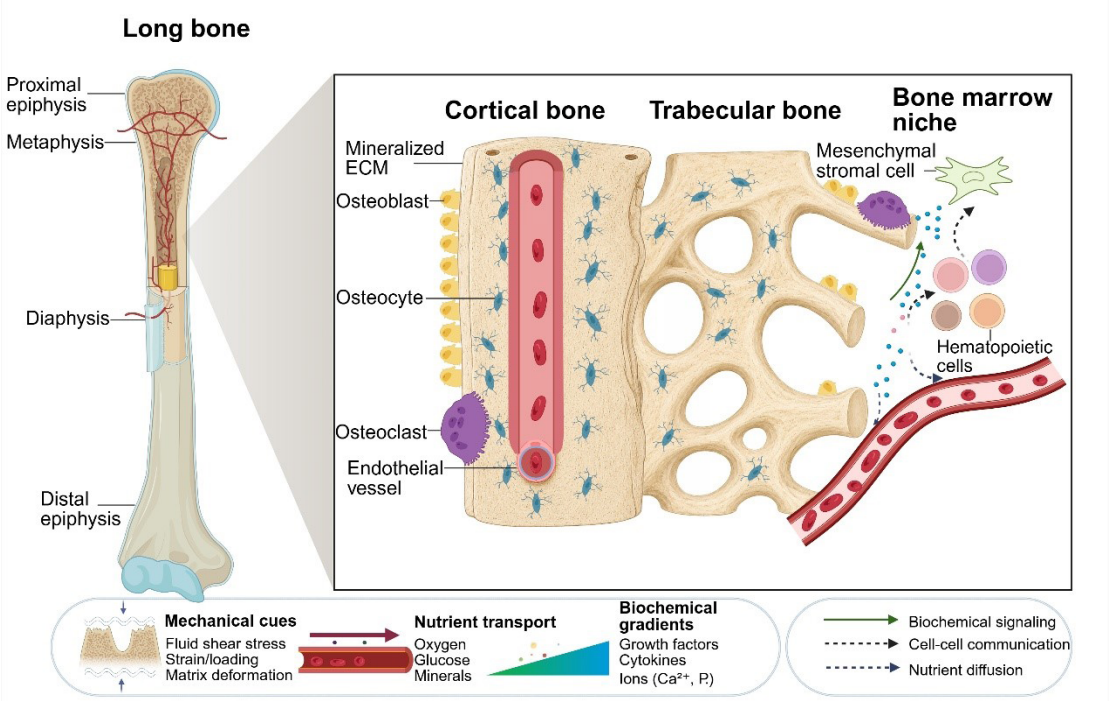


Figure 1. Structural and cellular organization of the native bone microenvironment. The schematic shows the hierarchical organization of long bone, including cortical bone, trabecular bone, and the bone marrow niche, as well as key bone-resident and marrow-associated cells. Mechanical cues, nutrient transport, biochemical gradients, and intercellular signaling collectively regulate bone remodeling and homeostasis. These features provide biological targets for the design of engineered bone organoids and bone-on-chip systems.

architecture, matrix composition, biochemical gradients, perfusable conduits, and loading conditions as tunable variables. The following sections discuss how organoid systems meet these requirements biologically and how 3D printing and chip technologies enable their reconstruction to be more controllable and reproducible. The structural and cellular organization of the bone microenvironment is schematically illustrated in Figure 1. The major material, cellular, biochemical, and physical components of the bone microenvironment are summarized in Table 1.

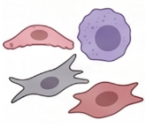
3. Organoid-based biomimicry of the bone microenvironment

Bone organoids biomimic the skeletal niche by reconstructing the defining features of bone as a mineralized, vascularized, and mechanosensitive tissue. Unlike most soft-tissue organoids, they must reproduce not only an osteogenic and mineralization-permissive matrix, but also multicellular remodeling crosstalk, transport competence, and mechanically regulated signaling.[11,12,21,22] At the matrix level, this is achieved by combining mesenchymal stromal cells or pluripotent stem cell-derived osteogenic lineages with hydrogels or composite biomaterials enriched with bioceramics, bone-derived ECM components, or ion-releasing materials, thereby supporting cell adhesion, matrix deposition, and ordered biomineralization.[12,21,23] At the cellular level, bone organoids re-establish interactions among osteoblast-lineage cells, osteocyte-like cells, endothelial cells, stromal cells, and, in more advanced models, hematopoietic or immune-related components, which are essential for coupled remodeling, osteogenic maturation, osteocyte network formation, and disease-relevant signaling. Their value, therefore, lies in guided self-organization, increasingly enabled by biomaterials, 3D bioprinting, and organ-on-chip technologies, and further strengthened by emerging platforms that integrate bone and marrow-like compartments.[18,20-23]

A further dimension of biomimicry is vascularization and dynamic transport. Because native bone is highly vascularized, static bone organoids are often constrained by diffusion-limited nutrient supply, inadequate waste removal, hypoxia, and incomplete maturation.[29,30] Therefore, faithful biomimicry requires models with transport competence and vascular-associated regulation, making vascularization a core design requirement rather than a secondary optimization step.[23,29-34]

Mechanical biomimicry is equally essential because bone is a prototypical mechanosensitive organ.[25,26] *In vivo*, osteoblasts and osteocytes respond to matrix

Table 1. Compositional summary of the bone microenvironment

Bone microenvironment component	Material composition category	Representative components	Main functions
 Mineral phase	Inorganic component	Carbonate-substituted hydroxyapatite nanocrystals	Provides stiffness, mineralization, and compressive strength
 Organic matrix	Organic component	Type I collagen fibrils and lamellae	Provides toughness, structural framework, and support for mineral deposition
 Bone resident cells	Cellular component	Osteoblasts, osteoclasts, osteocytes	Regulates bone formation, resorption, remodeling, and mechanosensation
 Supporting stromal and vascular cells	Cellular component	Endothelial cells, mesenchymal stromal cells, hematopoietic cells	Supports angiogenesis, stromal regulation, hematopoietic niche, and microenvironment homeostasis
 Soluble biochemical factors	Biochemical signaling component	Growth factors, chemokines	Regulates proliferation, differentiation, migration, angiogenesis, and tissue repair
 Biophysical regulatory environment	Physical/functional component	Mechanical cues, transport gradients, vascular perfusion	Regulates mechanotransduction, nutrient/waste transport, and dynamic microenvironmental control

stiffness, compression, tension, and interstitial fluid shear, and these cues shape osteogenic commitment and the balance between bone formation and resorption. Bone organoid systems increasingly address this requirement by integrating self-organized tissues with mechanically defined biomaterials, perfusion bioreactors, and chip-based loading systems.[22,35,36] Here, 3D bioprinting is especially valuable because it allows pore architecture, regional stiffness, and spatial organization to be specified in advance.[13,21,37]

Self-organization provides the basis for tissue-like assembly, but in bone-like systems, it is rarely sufficient to ensure stable mineralization, transport competence, vascular integration, or mechanical relevance.[11,12] 3D bioprinting and organ-on-chip technologies help convert these requirements into controllable structural, material,

and functional parameters.[18,20-22] Figure 2 summarizes how organoid-based systems biomimetically reconstruct the matrix, vascular, cellular, and mechanical features of the bone microenvironment.

4. Three-dimensional printing-enabled programmable reconstruction of the bone microenvironment

Reconstructing the bone microenvironment requires coordinated control of scaffold architecture, mineralization-permissive matrices, vascular networks, perfusion, and mechanical stimulation, with increasing attention to immune and neural regulation.[35,38,39] In this context, the value of 3D printing lies in its ability to convert these determinants into controllable and reproducible design parameters. From this perspective, programmable reconstruction of the bone microenvironment can be understood in terms of four interrelated aspects: printable biomaterial design, printable structural design, integration of biological signals, and current limitations.

More specifically, 3D bioprinting contributes to the development of bone organoids and bone-on-chip systems in several ways. First, it enables spatial positioning of osteogenic, endothelial, stromal, and immune-related cells and organoid-like modules within predefined architectures, thereby avoiding the limitations of random cell mixing. Second, it allows reproducible programming of matrix composition, pore geometry, stiffness, and mineral distribution, which is essential for reconstructing mineralized, mechanically relevant bone-like tissues. Third, sacrificial, coaxial, and multi-material printing strategies can generate hollow or perfusable channels that facilitate vascular-like transport and later integration with microfluidic chips. Fourth, printed structures can define force-transmission pathways and mechanically active interfaces, thereby enabling a more controlled application of compression, tension, or fluid shear. Finally,

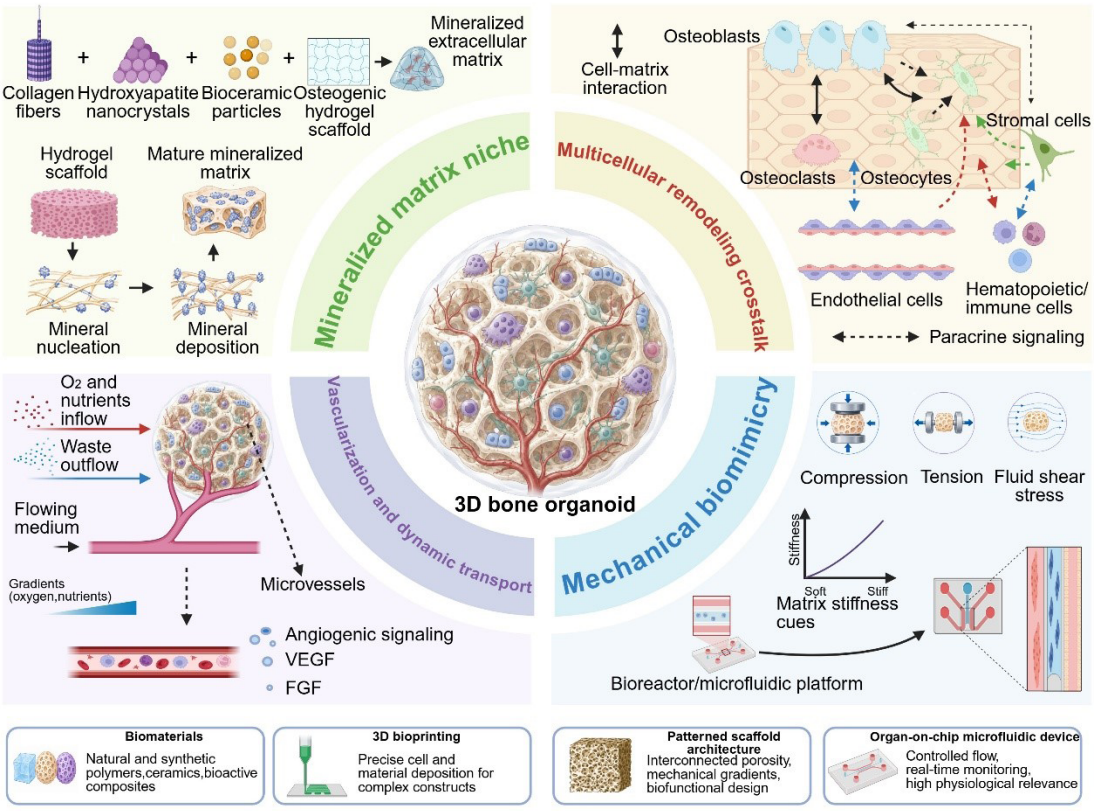


Figure 2. Organoid-based biomimetic reconstruction of the bone microenvironment. The schematic summarizes four core design requirements for bone organoid systems: mineralized matrix formation, multicellular remodeling crosstalk, vascularization and dynamic transport, and mechanical biomimicry. Biomaterials, 3D bioprinting, patterned scaffold architectures, and microfluidic or bioreactor platforms are shown as engineering strategies that translate these biological requirements into controllable structural, cellular, and functional parameters.
Abbreviations: 3D: Three-dimensional; FGF: Fibroblast growth factor; VEGF: Vascular endothelial growth factor.

3D bioprinting can assemble spheroids or organoid-like building blocks into organized tissue patterns, combining self-organization with engineered reproducibility. In this sense, 3D bioprinting provides the structural and spatial bridge between biological organoid formation and dynamic organ-on-chip regulation. The overall logic of programmable bone microenvironment reconstruction through 3D printing and chip integration is summarized in Figure 3.

4.1. Engineering printable biomaterials for bone microenvironment reconstruction

Bone differs fundamentally from most soft-tissue organoid systems because it requires a rigid, mineralized, and porous ECM.[5,13] Accordingly, scaffold materials for bone organoid engineering must provide not only mechanical support and a permissive 3D niche for cell adhesion, growth, and lineage commitment,[40] but also pathways for nutrient diffusion and vascular ingrowth.[38,40] In 3D printing, these features are not merely material constraints; they become programmable fabrication variables.

Material selection should therefore be evaluated based on printability, osteoconductivity, geometric fidelity, and compatibility with long-term remodeling. Current strategies combine hydrogels with bioceramics, bone-mimetic inorganic phases, and functional nanocomponents to generate composite bioinks that provide structural stability and support osteogenic maturation.[41-44] For example, gelatin methacryloyl/alginate methacrylate/hydroxyapatite systems have been shown to support apatite deposition while improving mineralization and mechanical performance.[44]

This material-level programmability is especially important for matrix mineralization. Native bone matrix is formed through the coordinated assembly of collagen fibrils and hydroxyapatite crystals,[45] and failure to reproduce this process compromises phenotypic fidelity and reduces utility for drug testing and biomaterial evaluation.[46,47] Importantly, ordered mineralization is not equivalent to nonspecific calcification,[12] but depends on coordinated regulation of ion availability, nucleation sites, collagen-templated crystal growth, alkaline enzymatic activity, transport, and mechanical boundary conditions.[35,48,49] Thus, 3D printing shifts mineralization from a passive bulk-material property toward a programmable outcome of scaffold design.

Composite bioinks exemplify this shift. Incorporation of nano-hydroxyapatite or β-tricalcium phosphate can increase stiffness while providing nucleation sites and sustained calcium/phosphate release.[50,51] Other nanocomponents, such as graphene oxide and zeolitic imidazolate framework-8, further regulate surface energy,

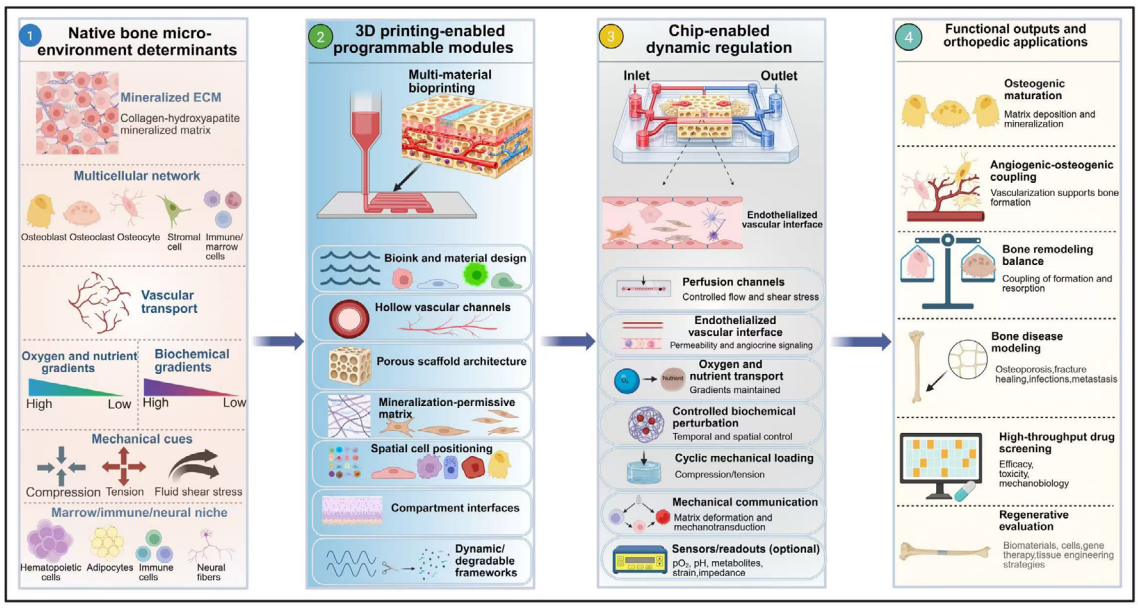


Figure 3. Programmable reconstruction of the bone microenvironment through 3D bioprinting and chip integration. The schematic shows how native bone microenvironmental determinants are translated into printable modules, including bioink/material design, vascular channels, porous architectures, mineralization-permissive matrices, spatial cell positioning, compartment interfaces, and dynamic or degradable frameworks. Chip-based regulation further introduces perfusion, vascular interfaces, biochemical perturbation, mechanical loading, and optional sensor-based readouts. These integrated systems support osteogenic maturation, vascular coupling, remodeling analysis, disease modeling, drug screening, and regenerative evaluation.
Abbreviations: 3D: Three-dimensional; ECM: Extracellular matrix.

protein adsorption, cell adhesion, and ion-release behavior, thereby influencing osteogenic signaling and long-term culture stability.[52,53] In parallel, self-mineralizing bioink designs can modulate diffusion and local supersaturation by tuning polymer ratios and crosslinking density, making mineralization onset and deposition more predictable.[44]

Therefore, printable biomaterial design is central not only to scaffold formation, but also to the reconstruction of a bone-like matrix with biologically meaningful mineralization. Key design aspects of 3D printing-enabled programmable scaffolds for bone microenvironment reconstruction are summarized in Table 2.

Table 2. Key design aspects of 3D printing-enabled programmable scaffolds for bone microenvironment reconstruction

Design aspect	Representative added contents	Main function	Advantage	Limitation	Representative references
Printable matrix phase	GelMA, alginate/AlgMA, collagen- or dECM-like components	Provide a printable 3D matrix for cell encapsulation, adhesion, and early tissue assembly	Good printability and cytocompatibility	Weak mechanical strength and limited osteoconductivity when used alone	40,41,44,54
Mineral reinforcement	HAP/nHAP, beta-TCP, calcium phosphate phases	Promote osteoconductivity, stiffness, and mineralized ECM formation	Improves bone-mimetic matrix reconstruction	Excess inorganic loading may impair printability, cell viability, and vascular infiltration	13,41,44,50,51
Bioactive nanocomponents	Graphene oxide, MOF/ZIF-8, ion-releasing particles	Enhance surface bioactivity, osteogenic signaling, and local mineralization-related microenvironment	Adds multifunctionality beyond mechanical support	Biosafety, dispersion stability, and reproducibility remain concerns	52,53,55
Structural and gradient programmability	Controlled pore architecture, porosity gradients, stiffness/composition gradients	Regulate nutrient transport, stress distribution, and spatial maturation	Better mimics hierarchical and heterogeneous bone structure	Difficult to simultaneously optimize fidelity, mechanics, and permeability	13,42,56-59
Cellular and biochemical integration	MSCs, endothelial cells, spheroids/organoid modules, osteogenic/angiogenic factors	Reconstruct the multicellular niche and localized instructive signaling	Improves osteogenesis, vascularization, and biological relevance	Increased fabrication complexity and limited long-term control of factor release	31,55,60-62
Dynamic and responsive design	Degradable guidance frameworks, responsive biomaterials, and 4D-printing elements	Support staged maturation, adaptive remodeling, and progressive self-organization	Better matches the developmental progression of bone-like tissues	Material design is complex, and standardization remains limited	59,63-65

Abbreviations: 3D: Three-dimensional; 4D: Four-dimensional; AlgMA: Alginate methacrylate; dECM: Decellularized extracellular matrix; GelMA: Gelatin methacryloyl; HAP: Hydroxyapatite; MOF: Metal–organic framework; MSC: Mesenchymal stem cell; TCP: Tricalcium phosphate; ZIF-8: Zeolitic imidazolate framework-8.

4.2. Printable structural design: architecture and fabrication strategy

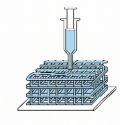
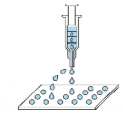
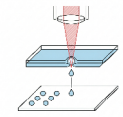
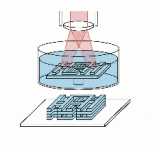
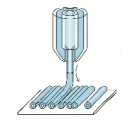
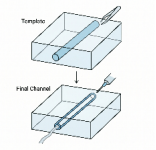
Beyond materials, 3D printing enables programmable structural design of bone-like tissues. A major advantage of bioprinting is its ability to define pore architecture, regional stiffness, filament arrangement, interface geometry, and compartmental organization, thereby creating constructs that better reproduce the heterogeneity of native bone.[13,41,42] This is particularly important for reconstructing trabecular-like regions, cortical-to-marrow transitions, and osteochondral or marrow-associated interfaces.

At the structural level, printing also enables mineralization-relevant gradients rather than uniform matrices. Through computer-aided design and multi-material deposition, scaffolds can be fabricated with continuous variations in porosity, stiffness, and composition, thereby emulating hierarchical bone

organization and transition zones.[13,37,56] These gradients influence oxygen and nutrient transport, ion diffusion, local supersaturation, stress distribution, and cell spreading, thereby shaping spatial mineralization patterns and lineage commitment.[57,58] For example, denser cortical-like regions may promote osteogenic commitment and mineralization, whereas more porous regions favor vascularization and marrow-like colonization.[13]

Different bioprinting modalities support these structural goals in complementary ways. Extrusion-based printing remains the most widely used in bone tissue engineering because it accommodates viscous, cell-laden, and ceramic-reinforced bioinks. Inkjet printing is better suited for localized deposition of cells or soluble biomolecules, whereas laser-assisted bioprinting enables high-resolution placement of cells and materials for microscale interfaces. Photopolymerization-based approaches, such as stereolithography and digital light processing, provide high geometric fidelity for complex porous architectures, while coaxial/core-shell and sacrificial printing enable hollow and perfusable structures relevant to vascular-like channels and organ-chip integration. Thus, structural design in bone organoid engineering is not merely shape control, but a fabrication strategy for regulating transport, mineralization, interfaces, and mechanical microenvironments. The major 3D bioprinting modalities relevant to orthopedic organoid and organ-chip fabrication are listed in Table 3.

Table 3. Summary of major 3D bioprinting modalities for orthopedic organoid and organ-chip fabrication

Bioprinting modality	Diagram	Key features	Advantages	Limitations	Relevance	Reference
Extrusion		Continuous deposition of viscous bioinks	Compatible with composite and cell-laden bone bioinks	Limited resolution; shear-induced cell stress	Bulk bone organoids; mineralized constructs	21,37,41,44,50,51
Inkjet		Drop-on-demand deposition of low-viscosity materials	High throughput; precise cue patterning	Limited material viscosity range	Biochemical patterning; localized seeding	21,37,66
Laser-assisted		Nozzle-free high-resolution transfer	Precise cell/material placement	High cost; technical complexity	Microscale interface reconstruction	21,37,66
Photopolymerization		Light-based fabrication of photocurable bioinks	High fidelity; reproducible porous structures	Limited material choices; phototoxicity concerns	Standardized organoid templates; patterned bone matrices	21,37,66,67
Coaxial/core-shell		Multilayer and hollow structure printing	Compartmentalized architectures; vascular-like features	Complex parameter optimization	Osteogenic-endothelial coupling; hollow organoid constructs	21,31,32,61
Sacrificial		Removable template-based channel fabrication	Perfusable conduits; chip compatibility	Extra processing steps	Vascularized organoids; perfusion-integrated chips	31-34

4.3. Integration of biological signals to reconstruct the bone microenvironment

The reconstruction of bone microenvironment depends not only on material and structural design, but also on the organization of cellular and biochemical signals. Bone organoid systems must restore interactions among osteoblast-lineage cells, osteocyte-like cells, mesenchymal stromal cells, endothelial cells, and, in more advanced models, hematopoietic, osteoclast-related, or immune-associated populations. In this sense, scaffold optimization also becomes a question of cell–matrix matching and multicellular biofunctional integration.

A key contribution of 3D bioprinting is enabling spatially controlled cell incorporation rather than uniform cell mixing. By depositing bioinks in predefined architectures, printing can localize cell populations to regions with different stiffness, porosity, or mineralization permissiveness, thereby improving compatibility among osteogenesis, vascular support, and later remodeling.[60,68] Designs based on cellular spheroids or organoid building blocks are especially promising because they preserve endogenous cell–cell interactions, enhance local cell density, and improve remodeling potential relative to single-cell dispersions.[59,60] Printed lattices, wells, or temporary support frameworks can position these modules in defined spatial relationships, combining developmental self-organization with fabrication reproducibility.

Three-dimensional printing also enables biochemical patterning. Because bone development and regeneration are governed by spatially and temporally coordinated osteogenic, angiogenic, and remodeling-related cues, homogeneous medium supplementation is often insufficient. In contrast, printing can localize growth factors and biochemical gradients during fabrication, linking bioactive instruction directly to scaffold architecture.[59,60] This logic is further extended in four-dimensional (4D) printing and stimuli-responsive bioinks, which are particularly relevant because bone is a dynamically remodeling tissue rather than a static mineralized scaffold.[63-65] In this context, 4D printing refers to printed constructs that can undergo time-dependent changes in architecture, stiffness, porosity, degradation, or biochemical release in response to defined stimuli. Temperature-responsive materials may support shape adaptation or staged construct assembly; pH-responsive systems may be useful for modeling acidic microenvironments associated with inflammation, infection, or tumors; enzyme-responsive bioinks can mimic cell-mediated matrix degradation and remodeling; and mechanosensitive materials may couple external loading to changes in matrix organization, stiffness, or bioactive cue presentation. These properties could help model sequential processes such as early tissue assembly, vascular invasion, osteogenic maturation, osteoclast-associated resorption, and adaptive remodeling under mechanical stimulation. However, the use of responsive bioinks also introduces challenges, including limited control over response kinetics, potential cytotoxicity of functional components, difficulty in matching stimuli to physiological ranges, and poor standardization across platforms. Therefore, 4D printing should be viewed as a promising but still developing strategy for introducing temporal regulation into bone organoid and bone-on-chip systems.

4.4. Current limitations and challenges

Despite these advances, several challenges remain. First, although numerous printed systems support mineral deposition, authentic bone-like mineralization still requires precise coordination of collagen templating, crystal maturation, transport, and mechanical conditioning rather than simple calcification.[49,69] Second, structural reproducibility does not necessarily confer physiological complexity: integrating vascular perfusion, marrow colonization, cortical-to-trabecular transitions, and mechanically relevant loading within a single construct remains difficult.[37,56] Third, multicellular complexity remains incomplete, particularly with respect to osteoclast-mediated remodeling, hematopoietic interactions, and immune or neural regulation.[35,38,39] Fourth, a persistent tension remains between external programmability and developmental autonomy: excessive engineering may constrain self-organization, whereas insufficient control compromises reproducibility.[12,59,60,63-65] Finally, evaluation standards remain fragmented, and more rigorous multiscale assessment of mineralization, collagen organization, transport, and mechanical competence is needed to define truly biomimetic printed bone organoids.[40,70,71]

The root causes of these limitations are multifactorial. Tissue immaturity often results from insufficient culture duration, incomplete osteogenic and osteocytic differentiation, limited collagen organization, inadequate mineral crystal maturation, and the absence of sustained perfusion or mechanical conditioning. Feasible solutions include staged maturation protocols, longer-term perfusion culture, osteocyte-supportive matrices, defined osteogenic cues, and dynamic mechanical stimulation matched to tissue-level functional readouts. An incomplete vascular hierarchy is related to simplified endothelial seeding, insufficient mural or stromal support, limited branching architecture, and incompatibility between endothelial and osteogenic culture requirements. This may be addressed by combining sacrificial or coaxial printing, prevascularized organoid modules, endothelial–stromal

co-culture, pericyte-like support, angiogenic gradients, and long-term controlled perfusion. Limited immune and hematopoietic integration reflects short immune-cell lifespan, phenotype drift, medium incompatibility, and difficulty maintaining immune–bone crosstalk. Modular immune or marrow compartments, timed immune-cell recruitment, hematopoietic or monocyte precursor reservoirs, and disease-specific inflammatory challenges may help improve physiological relevance. Finally, poor reproducibility arises from donor variability, bioink batch effects, printing-parameter sensitivity, and fragmented validation criteria. Automated fabrication, defined or xeno-free materials, standardized quality control, inline sensors, and shared benchmark assays will be important for making these platforms more reliable and comparable across studies.

Overall, 3D printing has transformed bone microenvironment reconstruction from empirical scaffold building into a more programmable strategy. However, achieving physiologically relevant bone organoid systems will require further advances not only in matrix authenticity, multicellular integration, mechanobiological regulation, and standardized evaluation, but also in dynamic microenvironmental control. These remaining needs provide a strong rationale for integrating printed constructs with chip-based platforms.

4.5. Validation standards for three-dimensional bioprinted bone organoid and bone-on-chip systems

Because bone organoid-on-chip platforms are designed for different applications, their validation should not rely on a single marker, staining method, or endpoint assay. Instead, a fit-for-purpose validation framework is required that integrates structural, molecular, functional, and reproducibility-related readouts. For 3D-bioprinted bone organoids and bone-on-chip systems, five domains are particularly important: mineralization, vascular perfusion, mechanical competence, osteoclast-mediated remodeling, and immune or osteoimmune function, as summarized in Table 4.

5. Multi-biomimetic chip systems for dynamic bone microenvironment modeling

Programmable scaffolds and bioprinting establish the structural and spatial basis for engineered bone-like tissues, but, by themselves, do not reproduce the dynamic regulation required for organoid maturation and function. In orthopedic organoid systems, tissue development also depends on continuous transport, mechanical stimulation, and niche-level crosstalk. Chip platforms, therefore, enable 3D-printed organoids to transition from static constructs into dynamically regulated microphysiological models. The main functional modules of multi-biomimetic chip systems for dynamic bone microenvironment modeling are illustrated in Figure 4.

5.1. Perfusable vascularized chip systems

Among the dynamic functions required for orthopedic organoid maturation, perfusion and vascularization are among the most fundamental. Bone is highly vascularized, and diffusion-limited static culture is often insufficient for maintaining viability, maturation, and functional complexity in larger constructs.[29,30] Perfusable chip systems matter not only because they improve medium exchange, but because they introduce controlled flow, endothelialized interfaces, and spatially defined transport pathways that reshape organoid survival, differentiation, and niche behavior.

Three-dimensional printing is central to this transition because it allows vascularization to be engineered as a predefined architectural feature rather than left entirely to spontaneous self-assembly. Hollow channels, sacrificial templates, and printed compartment interfaces can be incorporated during fabrication and later coupled to microfluidic perfusion modules, standardizing channel geometry, branching patterns, and chip-port connectivity.[31-33] In orthopedic organoid systems, vascularization is not only a support function but also a determinant of osteogenic maturation, endothelial–stromal crosstalk, and disease-relevant transport phenomena.

Prevascularization provides another route to improve network establishment and reduce the early hypoxic window.[61,72] Spheroids containing microvascular fragments can reconstruct networks after fusion,[73] but stable perfusion usually still benefits from printed support structures. Printed wells or lattices can position microtissues and constrain fusion topology, while printed trunk channels and manifolds standardize flow collection and chip-port interfaces.[74-76] Gradient-based vascular guidance can be further strengthened by combining angiogenic or oxygen gradients with printed resistance networks, diffusion barriers, and gradient generators.[34,62,77,78]

For disease modeling, vascularized chip systems contribute more than improved viability. They can reveal changes in endothelial permeability, barrier responsiveness, intravascular drug delivery, tissue penetration, and transendothelial transport.[79-81] Bone disease and tumor-related systems have shown that osteoporotic-like conditions can increase endothelial permeability and potentially facilitate tumor cell extravasation and growth.[82] Remaining limitations include insufficient vascular

Table 4. Validation standards for 3D-bioprinted bone organoid and bone-on-chip systems

Validation domain	Representative readouts	Minimum functional benchmark	Key interpretation/caveat
Mineralization	Alizarin Red S and von Kossa staining; calcium/phosphate quantification; micro-CT or SEM/EDX analysis; collagen organization; osteogenic markers such as RUNX2, ALPL, COL1A1, OCN, and OPN.	Evidence of organized bone-like mineral deposition together with osteogenic maturation and matrix organization.	Validation should distinguish biologically regulated bone-like mineralization from nonspecific calcium precipitation or dystrophic calcification.
Vascular perfusion	Endothelial markers such as CD31, VE-cadherin, and vWF; lumen formation; dextran or bead perfusion; permeability assays; oxygen/nutrient transport; response to flow or angiogenic stimulation.	Presence of endothelialized, perfusable, and transport-competent channels or vascular-like networks with measurable barrier or permeability properties.	Endothelial marker expression alone is insufficient; vascular validation should demonstrate functional perfusion and transport.
Mechanical function	Compressive or tensile modulus; rheology or nanoindentation; defined cyclic compression, tension, or fluid shear stress; internal strain transmission; mechanotransduction markers such as SOST, Wnt/β-catenin, YAP/TAZ, and PIEZO1.	Reproducible mechanical stimulation should be transmitted into the construct and induce tissue-level mechanotransduction or remodeling-related responses.	Mechanical validation should confirm the biological response to loading rather than merely confirm device actuation or scaffold stiffness.
Osteoclast-mediated remodeling	TRAP-positive multinucleated cells; osteoclast markers including CTSK, NFATC1, MMP9, and DC-STAMP; RANKL/OPG responsiveness; resorption-pit formation; formation–resorption coupling readouts.	Evidence of osteoclast differentiation, resorptive activity, and coupling with osteoblast- or osteocyte-lineage cells.	Osteoclast validation requires functional evidence of resorption, not just expression of osteoclast markers.
Immune/osteoimmune function	Viability and persistence of macrophages or other immune cells; M1/M2-related markers; cytokines such as TNF-α, IL-1β, IL-6, IL-10, and TGF-β; inflammatory challenge response; immune–osteoblast/osteoclast crosstalk.	Controlled inflammatory or osteoimmune responses that are relevant to the intended disease model, such as infection, osteolysis, arthritis, or tumor–bone interaction.	Immune validation should align with the disease context and avoid adding immune cells without verifying their phenotype, persistence, and functional crosstalk.

Abbreviations: ALPL: Alkaline phosphatase; CD31: Cluster of differentiation 31; COL1A1: Collagen type 1 alpha 1 chain; CT: Computed tomography; CTSK: Cathepsin K; DC-STAMP: Dendritic cell-specific transmembrane protein; EDX: Energy-dispersive X-ray spectroscopy; IL: Interleukin; MMP9: Matrix metalloproteinase 9; NFATC1: Nuclear factor of activated T cells 1; OCN: Osteocalcin; OPG: Osteoprotegerin; OPN: Osteopontin; PIEZO1: Piezo-type mechanosensitive ion channel component 1; RANKL: Receptor activator of nuclear factor κB ligand; RUNX2: Runt-related transcription factor 2; SEM: Scanning electron microscopy; SOST: Sclerostin; TAZ: Transcriptional coactivator with PDZ-binding motif; TGF: Transforming growth factor; TNF: Tumor necrosis factor; TRAP: Tartrate-resistant acid phosphatase; VE: Vascular endothelial; vWF: von Willebrand Factor; Wnt: Wingless/integrated; YAP: Yes-associated protein.

hierarchy, incomplete endothelial maturation, and the practical complexity of long-term perfused culture.

5.2. Mechanically active chip systems

Mechanically active chip systems are indispensable for reproducing the functional logic of the bone microenvironment. Osteoblasts and osteocytes respond to stretching, compression, and fluid shear, and these cues regulate osteogenic commitment and remodeling balance.[6,83,84] Within 3D-printed systems, mechanical stimulation should be understood as the integration of manufacturable interfaces, defined structural carriers, and controllable actuation.[36] Printing provides load-bearing geometries and reproducible force-transmission pathways, while chip platforms add stable perfusion and actuation.

In many bone-on-chip systems, flow-induced shear is the most commonly implemented cue.[85] Continuous perfusion generates microcirculatory-like wall shear stresses that influence endothelial and bone-related cells alike.[86] Because printing defines microchannel cross-sections, resistance networks, narrow gaps, and pore connectivity, shear distributions become functions of structure rather than solely of user-selected flow rate, improving reproducibility and cross-study comparability.[34] Related studies indicate that constrained, canaliculi-like flow environments can support osteocyte network establishment and phenotypic stability, while shear can promote dendritic process extension, network connectivity,

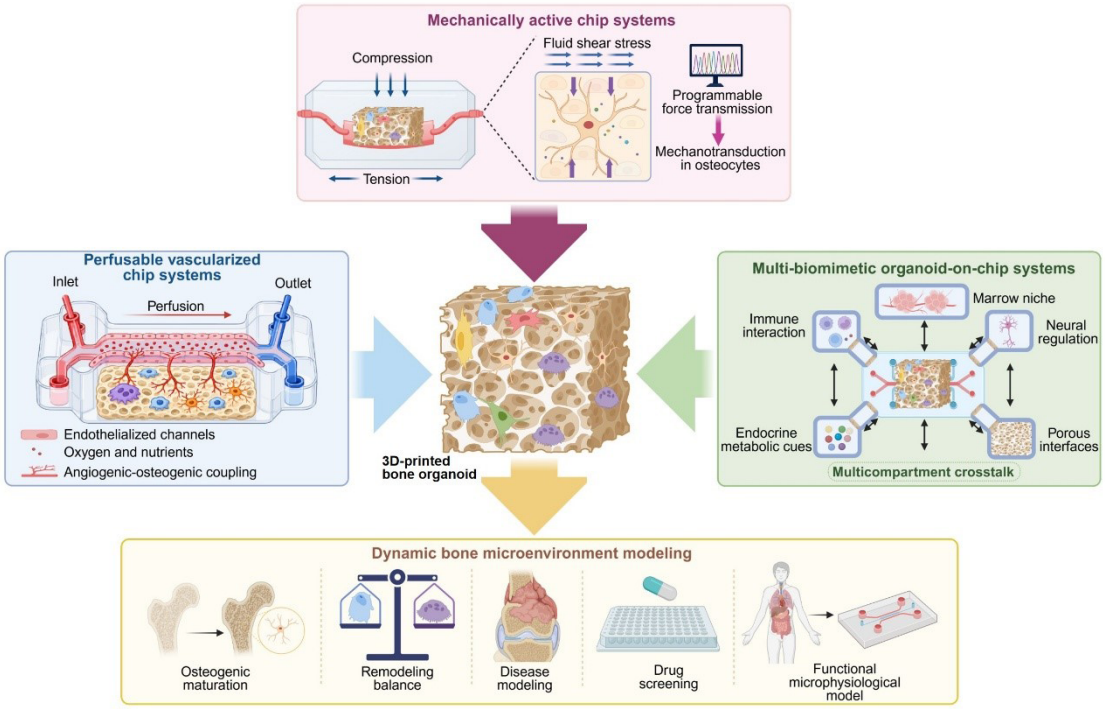


Figure 4. Chip-integrated dynamic modeling of the bone microenvironment. Perfusable vascularized chips, mechanically active chips, and multi-biomimetic organoid-on-chip systems provide dynamic regulation through perfusion, mechanical stimulation, vascular interfaces, and multicompartment crosstalk. These platforms support functional assessment of osteogenic maturation, remodeling balance, disease modeling, drug screening, and microphysiological relevance.
Abbreviation: 3D: Three-dimensional.

and mechanosensitive signaling such as sclerostin-related responses.[87,88]

Compression and tensile loading are also highly relevant because they correspond more directly to motion and load-bearing states.[22,89] Chip platforms can impose cyclic deformation through flexible membranes or deformable chambers, but the challenge in bone organoid contexts is transmitting these stimuli into the interior of a 3D tissue in a repeatable way.[89] Printed support frames, compressible pillar arrays, and matrices with defined stiffness can help convert externally imposed displacement into predictable internal strain fields.[90] Validation should then relate loading parameters to tissue-level readouts rather than simply confirming that loading occurred. Typical outputs include alkaline phosphatase activity, mineral deposition, Wingless/integrated signaling, and Piezo-type mechanosensitive ion channel component 1-associated mechanotransduction.[91,92] More broadly, evidence from printed cartilage systems suggests that appropriate structural constraints and maturation conditions can improve tissue-level mechanics and histology, supporting the broader principle that engineered boundary conditions matter for mechanically regulated tissue development.[93]

Mechanically active chip systems are most informative when coupled with scaffold architecture and perfusion, because geometry shapes force transmission and shear distribution, and actuation defines stimulus waveform. This integration becomes even more important once immune, neural, marrow, and endocrine influences are incorporated.

5.3. Multi-biomimetic organoid-on-chip systems for dynamic orthopedic microenvironment modeling

The native bone microenvironment extends far beyond osteogenic cells and vascular endothelium. It includes marrow hematopoietic populations, immune cells, nerve fibers, and periosteal fibroblasts, all of which contribute to niche regulation and remodeling.[94] In disease settings such as rheumatoid arthritis, osteomyelitis, and inflammatory bone destruction, these non-osteogenic components become central drivers of pathology.[95] Accordingly, advanced chip systems should be designed as multicompartment platforms in which these influences can be introduced modularly and studied under controlled conditions.

Spatial compartmentalization and tunable porosity

provide a practical route for implementing such modules.[96] Printed constructs can define compartments or graded regions that support timed recruitment or co-culture of macrophages, T cells, and related populations, enabling mechanistic analysis of immune–osteoblast–osteoclast interactions.[97] Multi-material printing and hollow-cavity architectures can also support bone–marrow partitioning, with hematopoietic stem or progenitor cells seeded into inner luminal spaces to create marrow-like communities.[98,99]

Neural regulation can likewise be introduced through neuronal explants, neural cell populations, or localized neurotrophic cues, which is relevant given the close link between bone remodeling, pain, and peripheral sensory innervation.[100] Endocrine and metabolic influences can be incorporated as non-cellular modules through controlled medium formulations and perfusion conditions, including estrogen withdrawal or high-glucose environments relevant to osteoporosis and metabolic bone disease.[96,101]

Although immune integration remains relatively limited in engineered bone systems,[84] microfluidic periodontal disease platforms have already demonstrated feasible routes for coupling tissue, microbiome, and immune interactions under controlled perfusion and for observing chronic inflammation-driven bone resorption.[102-104] These concepts are directly relevant for modeling rheumatic disease and osteomyelitis.[105,106] At the same time, increased complexity introduces limitations, including short immune cell lifespan, demanding maintenance, difficulty in assigning causality across coupled interactions, and the lack of shared standards for benchmark comparison.[107,108] Even so, the structural freedom provided by 3D printing creates a realistic path toward incorporating immune and neural regulation in ways that may improve prediction of global drug efficacy and adverse effects, especially for pathways such as receptor activator of nuclear factor κB ligand (RANKL) signaling that bridge bone and immunity.[109-111]

5.4. Toward integrated organoid-on-chip platforms

Taken together, multi-biomimetic chip systems show that 3D bioprinting functions as a microenvironment modularization platform as much as a structural fabrication method.[21,112] Supporting precise spatial fabrication and integration with vascular, mechanical, inflammatory, neural, marrow, and endocrine modules allows bone microenvironment models to be reconfigured for distinct biological questions and disease contexts.

This design-to-disease logic is essential because different orthopedic applications require different combinations of platform parameters and validation endpoints. Bone defect models prioritize mineralized and mechanically competent scaffolds, vascularized osteogenesis, defect-matched geometry, and integration trajectories. Osteoporosis models require formation–resorption balance, osteoblast–osteoclast–osteocyte readouts, osteoclast support, and endocrine or metabolic perturbation. Osteoarthritis models require osteochondral gradients, compartmentalization of the cartilage–bone–synovium, inflammatory stimulation, and cyclic mechanical loading. Bone tumor and metastasis models require tumor–bone interfaces, vascular barriers, invasion routes, permeability readouts, and longitudinal drug-response assessment. Therefore, the following section discusses orthopedic applications based on how platform parameters are matched to disease-specific biological requirements.

6. Construction and application of orthopedic tissue regeneration and disease models

The 3D-printed bone organoids and organoid-on-chip systems are increasingly being used as disease-relevant testbeds that bridge the gap between reductionist cell culture and animal models. Their value lies in combining 3D cell–cell and cell–matrix interactions with controllable inputs such as perfusion, mechanics, and gradients, while providing clinically meaningful readouts in a standardized format. The following sections highlight representative applications across major orthopedic indications. A conceptual framework linking 3D-printed organoids and organoids-on-chip to representative orthopedic research applications is shown in Figure 5. A fit-for-purpose assembly of organoid and chip modules across representative orthopedic applications is provided in Table 5.

Compared to conventional 2D culture, 3D-bioprinted bone organoids and bone-on-chip systems better preserve 3D cell–cell and cell–matrix interactions, spatial gradients, mineralized matrix organization, and multicellular crosstalk. In contrast to static 3D cultures, chip-integrated platforms further introduce controlled perfusion, compartmentalized communication, mechanical stimulation, and real-time or longitudinal readouts. For animal models, these systems provide more controllable, human-relevant, and potentially higher-throughput platforms for mechanistic studies and drug evaluation, although they still cannot fully reproduce systemic endocrine, immune, metabolic, and load-bearing conditions *in vivo*. Therefore, their value should be understood in a fit-for-purpose manner: bone defect models are useful for evaluating vascularized osteogenesis and integration trajectories; osteoporosis models allow combined assessment of formation–resorption balance;

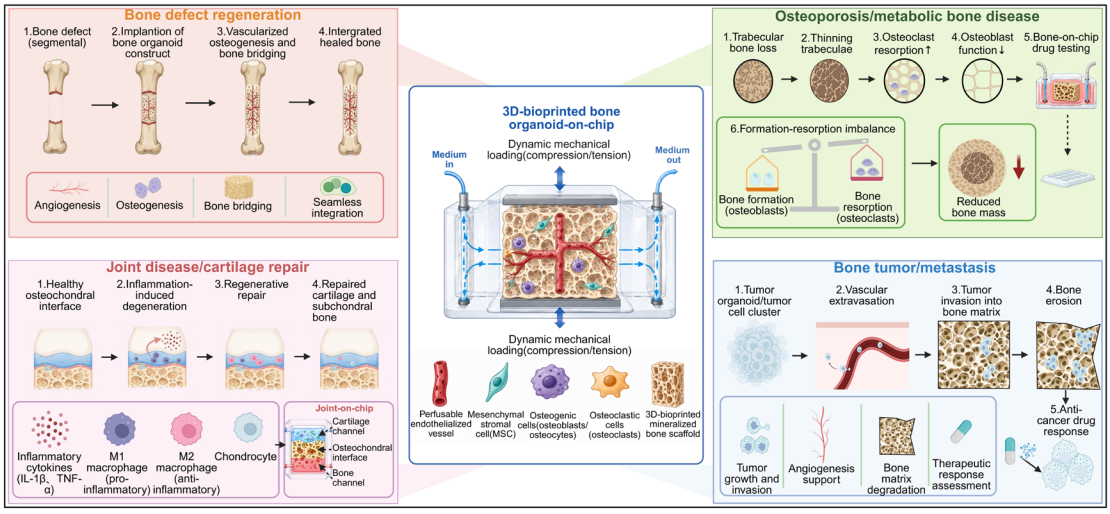


Figure 5. Applications of 3D-bioprinted bone organoid-on-chip systems in orthopedic research. The schematic links a central 3D-bioprinted bone organoid-on-chip platform to major orthopedic applications, including bone defect regeneration, osteoporosis and metabolic bone disease, joint disease and cartilage repair, and bone tumor or metastasis. Disease-relevant processes and functional outputs are highlighted, such as vascularized osteogenesis, bone bridging, formation–resorption imbalance, osteochondral inflammation and repair, tumor invasion, bone erosion, and therapeutic response assessment.

Abbreviations: 3D: Three-dimensional; IL: Interleukin; TNF: Tumor necrosis factor.

osteoarthritis models help reconstruct cartilage–bone–synovium interactions under inflammatory and mechanical cues; and tumor or metastasis models enable controlled analysis of tumor–bone remodeling, vascular permeability, invasion, and therapeutic response.

6.1. Bone defect and bone tissue engineering

Large segmental defects caused by trauma, infection, or tumor resection remain a persistent challenge in orthopedics.[113] Autografts and allografts are still widely used, yet both are constrained by limited availability, donor-site morbidity, and variable outcomes.[114] Bone tissue engineering has therefore focused on integrating cells, scaffolds, and instructive factors, but success remains strongly context dependent because vascular ingrowth kinetics, osteogenic cell competence, and evolving inflammatory and mechanical cues jointly shape healing trajectories.[115] Organoid and organ-on-chip strategies aim to reconstruct key phases of bone regeneration within controllable systems and to evaluate emerging interventions quantitatively.[20]

Recent work illustrates how spatial design can address biological bottlenecks in repair. Zhang *et al.*[116] assembled mesenchymal stromal cell–loaded cores enriched with osteogenic and neurotrophic cues and combined them with proangiogenic outer shells, implanting multiple units into a rat femoral segmental defect. This "divide-and-conquer" architecture accelerated bridging by promoting the formation and coalescence of ossified nodules, supporting the idea that spatially partitioned osteoinductive and angiogenic signaling can shorten the sequence from perfusion establishment to mineralized matrix deposition. In parallel, prevascularization has gained traction as a strategy to shorten the early hypoxic window after implantation.[117] Studies using microvascular fragments to biofabricate prevascularized spheroids support this rationale,[73] and related work combining prevascularized bone organoids with bioprinting has reported rapid *in situ* cranial bone reconstruction.[23] Beyond implantation-oriented demonstrations, organoid and chip platforms are also being adopted as screening tools for biomaterials, factor delivery schemes, and culture regimens.[86]

Despite clear progress, several barriers still limit current applications in bone regeneration. Many demonstrations still rely on rodent-derived cells and small-animal validation, leaving uncertainty about their human predictive value.[118] Broader use of human mesenchymal stromal cells and induced pluripotent stem cell-derived lineages within organoid and chip formats is therefore needed.[119] Scale and mechanics also remain unresolved; even when bridging is robust in proof-of-concept settings, reconstructing load-bearing bone or anatomically complex defects requires stronger mechanical stability and better control of final geometry.[120] Safety is another issue because multi-factor or multi-material constructs may increase immunogenicity risk or off-target ossification, highlighting the need for more comprehensive evaluation pipelines.[121]

Table 5. Fit-for-purpose assembly of organoid and chip modules across representative orthopedic applications

Model/ application	Cell source	Bioink/scaffold	Printing/chip design	Key readouts	Advantages	Limitations
Bone defect regeneration	MSCs, iPSCs/ osteogenic cells, endothelial cells, spheroids or organoid modules	GelMA/alginate/ collagen/dECM composites with HAP, nHAP, β-TCP, or ion-releasing phases	Porous lattices, mineral/stiffness gradients, osteogenic-angiogenic compartmentalization, perfusion channels	Mineralized ECM, vascular invasion, integration, and mechanical integrity	Defect-specific geometry and spatial osteogenic/ angiogenic programming	Scale-up, load-bearing realism, vascular hierarchy, and human predictiveness remain limited
Osteoporosis/ metabolic bone disease	Osteoblast-lineage cells, osteocytes, osteoclast precursors, endothelial/ stromal cells, patient/iPSC-derived cells	Mineralized hydrogel or scaffold supporting formation-resorption balance	Bone-on-chip or organoid-on-chip with perfusion, endocrine/metabolic perturbation	OB/OC/ OY markers, resorption pits, osteocyte network, permeability, drug response	Models coupled remodeling and separate the anabolic versus antiresorptive effects	Sustained osteoclast source, systemic endocrine regulation, and long-term stability are challenging
Joint disease/ cartilage repair	Chondrocytes, MSCs, osteogenic cells, synoviocytes, and inflammatory cells	Zonal cartilage-bone hydrogels, osteochondral composites, scaffold-free cartilage modules	Osteochondral gradients, cartilage-bone-synovium compartments, cyclic compression/ shear, inflammatory exposure	Cartilage degeneration, subchondral remodeling, cytokines, matrix mechanics	Captures multi-tissue crosstalk and mechanical/ inflammatory cues better than monoculture	Divergent culture requirements and the chronic disease timescale remain difficult
Bone tumor/ metastasis	Patient-derived or cell-line tumor organoids, osteogenic stromal cells, endothelial cells, osteoclast/ immune modules	Tumor/bone dECM, mineralized matrix, stromal hydrogels	Vascularized tumor-bone chips, invasion interfaces, endothelial barriers, and perfused drug delivery	Tumor growth/ invasion, bone erosion, permeability, dormancy markers, drug sensitivity	Enables real-time tumor-bone interaction and parallel therapeutic testing	Patient heterogeneity, immune deficiency, and dynamic tracking standards remain gaps
Bone infection/ osteomyelitis	Osteoblast-lineage cells, osteocytes, MSCs, macrophages, neutrophils, T cells, and microbial components such as S. aureus	Mineralized matrix, infected bone-like scaffold, biofilm-permissive surfaces, and immune-compatible culture conditions	Compartmentalized bone-immune-microbe chips, perfusion systems, antibiotic gradients, and controlled microbial exposure	Bacterial burden, biofilm formation, cytokine release, immune phenotype, osteoblast viability, osteoclast activation, bone resorption, antibiotic penetration	Models host-pathogen-bone interactions and support antibiotic or anti-inflammatory testing	Biosafety requirements, microbial standardization, immune-cell lifespan, and long-term co-culture remain challenging
Patient-specific orthopedic modeling	Patient-derived MSCs, iPSC-derived osteogenic cells, tumor organoids, immune cells, and disease-specific stromal cells	Patient-matched hydrogels, mineralized matrices, dECM-based scaffolds, or standardized screening-compatible biomaterials	Personalized organoid-on-chip systems, parallelized microfluidic arrays, and high-throughput drug-testing formats	Patient-specific mineralization, resorption, inflammatory response, tumor growth, drug sensitivity, toxicity, and clinical correlation	Supports individualized and mechanism-based testing beyond generic cell lines	Donor variability, inconsistent iPSC differentiation, limited clinical validation, long preparation time, high cost, and standardization barriers

Abbreviations: dECM: Decellularized extracellular matrix; ECM: Extracellular matrix; GelMA: Gelatin methacryloyl; HAP: Hydroxyapatite; iPSC: Induced pluripotent stem cell; MSC: Mesenchymal stem cell; OB: Osteoblast; OC: Osteoclast; OY: Osteocyte; TCP: Tricalcium phosphate.

Bone organoids also suggest a pathway toward more modular regenerative products. A plausible workflow involves manufacturing patient-specific organoids from autologous cells, cryopreserving them for on-demand use, and enabling in situ maturation with less reliance on high-dose exogenous growth factor delivery.[122] Realizing this vision will require closer integration between printed architectures, vascularization strategies, and chip-based assessment pipelines.[123]

6.2. Osteoporosis and metabolic bone diseases

Osteoporosis is characterized by reduced bone mass and deterioration of microarchitecture, leading to increased skeletal fragility.[124] Therapeutic development requires an integrated evaluation of drug effects on both formation and resorption.[47] Conventional preclinical models, including ovariectomized rodents, suffer from interspecies differences and often do not permit mechanistic interrogation of osteoblast–osteoclast coupling.[125] Therefore, organoids and microphysiological systems offer a promising approach to reconstruct human-relevant remodeling in vitro for mechanistic studies, personalized evaluation, and high-throughput screening.[126]

Representative platforms demonstrate how osteoporosis-oriented bone chips can connect remodeling state to disease outcomes. Lee *et al.*[82] established a bone organoid-on-chip that simulated normal, osteopenic, and osteoporotic conditions by modulating culture medium and cellular compositions. When breast cancer cells were introduced, tumor growth occurred in all groups but was markedly enhanced in the osteoporotic chips, accompanied by greater erosion of the bone niche. This work not only linked osteoporotic remodeling to metastatic progression *in vitro* but also highlighted how bone chips can be used to evaluate pharmacological efficacy under osteoporosis-relevant conditions. Complementing state-modeling chips, Lipreri *et al.*[127] developed a 96-well bone-on-chip screening platform focused on osteocyte network viability and function. Because osteocytes orchestrate the balance between osteoblast and osteoclast activity, this system enabled quantification of anabolic responses, including dendrite elongation, increased cellular activity, and changes in key proteins such as connexin 43 following treatment. Compared to conventional 2D osteoblast cultures, these organoid and chip-based screening formats better reflect the complexity of the *in vivo* microenvironment and can provide more informative early-stage readouts.[128]

Nevertheless, robust osteoporosis modeling remains challenging. Sustaining osteoclast activity is difficult because osteoclasts arise from the monocyte–macrophage lineage, have limited survival *in vitro*, and require continuous support from factors such as macrophage colony-stimulating factor and RANKL.[129] Incorporating hematopoietic stem or progenitor populations to replenish osteoclast precursors has been proposed as one solution.[130] In addition, major systemic drivers of osteoporosis—including endocrine regulation by estrogen and parathyroid hormone and metabolic influences related to vitamin D status and calcium–phosphate balance—are not captured in isolated bone constructs.[131] Multi-organ integration, such as coupled bone–liver–gut platforms, may therefore be needed to reproduce calcium handling and whole-body bone metabolism.[132] Patient-specific induced pluripotent stem cell (iPSC)-derived osteogenic organoids may help address disease heterogeneity.

Overall, organoid and chip technologies offer an unprecedented window into osteoporosis at cellular resolution. They enable more integrated interrogation of communication among osteoclasts, osteoblasts, and osteocytes,[101] and for drug evaluation, they can separate anabolic from antiresorptive actions by jointly tracking formation and resorption readouts rather than relying mainly on endpoint bone mineral density changes.[47,133] Such platforms may also support rational combination screening and, eventually, individualized therapy selection.[134]

In addition to mechanistic modeling, osteoporosis-oriented organoid and chip systems are increasingly being adapted for pharmacological screening and efficacy evaluation. Compared to conventional 2D cultures, these platforms permit concurrent assessment of osteogenic activity, osteoclastic resorption, and osteocyte network function, thereby enabling clearer discrimination between anabolic and antiresorptive effects.[47,84,127] Lipreri *et al.*[127] further demonstrated the screening potential of a 96-well bone-on-chip platform by quantifying osteocyte viability, dendritic morphology, and treatment-related molecular changes in a standardized format. Such designs also make higher-throughput testing more feasible and may improve early-stage compound triage by providing human-relevant, multiparametric readouts before animal studies.[135-138] In the future, patient-derived or iPSC-based osteogenic organoids may further extend this concept toward individualized testing of anti-osteoporotic interventions.[139,140]

6.3. Joint disease and cartilage repair

Osteoarthritis features progressive degeneration of articular cartilage, accompanied by pathological remodeling of subchondral bone.[141] Conventional animal models rarely reproduce the full spectrum of osteoarthritis in a single system, particularly the interplay among chronic inflammation, cartilage wear, and subchondral sclerosis.[142]

In parallel, joint tissue engineering aims to restore the osteochondral interface, which remains difficult because of strong gradients in stiffness, vascularization, and cellular composition.[143] Organoid and microphysiological system technologies offer an emerging route to construct joint-like models that integrate cartilage, synovium, and subchondral bone for studies of osteoarthritis pathogenesis and drug screening.[144]

Zhang *et al.*[145] reviewed strategies for small-joint organoids and highlighted key biological and engineering barriers. A central difficulty is that cartilage is avascular and aneural, limiting intrinsic repair capacity,[146] while recreating joint-level architecture and multi-tissue interfaces remains challenging.[147] Here, 3D bioprinting is valuable because it enables controlled design of organoid geometry, internal structure, mechanical properties, and spatial cell organization, supporting reconstruction of cartilage, subchondral bone, and osteochondral transition zones through anatomically informed constructs, zonal patterning, and coupled mechanical and chondrogenic cues.[148-150]

Progress in joint-on-chip systems further underscores the importance of integrating multiple tissues under controlled biophysical and biochemical regulation.[89] Roehm *et al.*[151] reported a joint-on-chip that combined cartilage, synovium, and bone within a microfluidic and mechanically integrated platform. By incorporating perfusion, cyclic compressive loading, and inflammatory stimulation, this system addressed limitations of static cultures and traditional models that do not adequately capture joint mechanics or immune-associated cues. In a complementary direction, Nonaka *et al.*[93] used Bio-3D printing to generate scaffold-free cartilage tissues from adipose-derived stem cells, supporting the concept that coupling self-organization with bioprinting can produce high-quality cartilage tissues without exogenous scaffolds.

Despite advances, substantial challenges remain for joint organoid and chip models. Recreating multi-tissue interfaces is difficult because cartilage, bone, and synovium differ markedly in stiffness, metabolic requirements, and optimal culture conditions.[143] Maintaining these divergent requirements in a shared system is nontrivial, and capturing the chronic tempo of osteoarthritis remains difficult because disease progression often exceeds typical *in vitro* culture windows.[152,153]

6.4. Bone tumor and bone metastasis

Primary bone malignancies, such as osteosarcoma and secondary skeletal tumors arising from metastasis, remain major threats to patient survival.[154] Dissecting microenvironmental regulation and therapeutic response requires models that capture bidirectional tumor–bone interactions, which are difficult to reproduce with conventional cell-line culture and many animal models. Integrating 3D-bioprinted tumor organoids with bone-on-chip platforms provides a more faithful framework for emulating tumor growth within bone, bone remodeling responses, vascular permeability, and treatment sensitivity.[155,156]

Puce *et al.*[157] emphasized that limited progress in osteosarcoma therapy is partly attributable to the poor predictive power of traditional models. 2D cultures and many *in vivo* systems fail to recapitulate intratumoral heterogeneity and the distinctive bone niche that shapes osteosarcoma behavior.[158] In contrast, 3D bioprinted organoids and organ-on-chip approaches can better approximate *in vivo* complexity, and both scaffold-free and scaffold-based spheroid formats can establish hypoxic gradients and relevant matrix dynamics.[155,159]

Several platforms highlight these advantages. Lu *et al.*[160] developed an osteosarcoma-on-chip that integrated tumor cells with bone matrix components and a microcirculatory environment. A 3D-printed microtumor assembled using decellularized osteosarcoma matrix and bone marrow-derived exosomes, followed by perfusion culture on a chip, enabled sustained growth and phenotypes more closely resembling *in vivo* behavior.[160] A customizable agarose micropatterned platform likewise generated reproducible osteosarcoma spheroids and supported automated imaging and drug-response assessment.[161] More broadly, organoid and chip technologies enable co-culture of tumor and bone organoids via microfluidic coupling, supporting mechanistic modeling of tumor-driven bone erosion and screening for agents that inhibit tumor invasion into bone.

Despite rapid progress, *in vitro* bone tumor modeling still faces key gaps. Tumor heterogeneity across patients motivates the development of organoid biobanks or diversified chip panels.[162] Spatiotemporal resolution is another limitation: bone invasion is dynamic, yet many studies emphasize endpoints such as tumor burden and bone loss, with limited real-time tracking of events, including osteoclast activation and dormancy programs.[163] Finally, immune regulation remains underrepresented in numerous current models, underscoring the need to incorporate relevant immune components to improve physiological fidelity and translational relevance.[164]

Beyond disease reconstruction, bone tumor and metastasis models are also emerging as practical platforms for drug screening, mechanism-of-action studies, and safety evaluation. By integrating tumor cells with stromal, vascular, and bone-specific components, these systems enable simultaneous monitoring of tumor growth,

microenvironmental remodeling, and treatment-induced changes in endothelial or bone-related markers, thereby offering a more informative assessment of therapeutic efficacy than conventional monoculture assays.[165,166] Their translational value is further supported by micropatterned spheroid platforms that facilitate reproducible osteosarcoma spheroid formation and standardized assessment of treatment responses.[161] In addition, patient-derived tumor organoids may retain key genetic and phenotypic features of the original lesion, creating opportunities for individualized drug sensitivity testing in aggressive bone malignancies and metastatic disease.[167,168]

6.5. Critical appraisal of negative, failed, and conflicting evidence

Despite rapid progress, current 3D-bioprinted bone organoids and bone-on-chip systems should be interpreted as fit-for-purpose models rather than complete replicas of native bone or orthopedic disease. Several commonly reported positive outcomes remain context-dependent and require careful interpretation. For example, mineral staining alone cannot distinguish organized bone-like mineralization from nonspecific calcification,[12,69] and endothelial marker expression or printed channels do not necessarily indicate mature, functionally perfusable vasculature.[61,81] Increasing ceramic content may improve stiffness and osteoconductivity but can compromise printability, cell viability, and vascular infiltration.[50,54] Mechanical stimulation may promote osteogenic maturation, but its effects vary with loading parameters, scaffold properties, and culture duration and should therefore be assessed using validated mechanotransduction and functional readouts.[35,89,92]

Disease-specific platforms also show substantial unresolved gaps. Bone defect models often remain proof-of-concept studies and lack validation under clinically relevant load-bearing conditions.[118,125] Osteoporosis models still require more stable osteoclast sources, systemic metabolic regulation, and improved patient-specific representation.[129,139] Osteoarthritis models require better long-term integration of cartilage, subchondral bone, synovium, inflammation, and mechanical loading.[143,152,153] Bone tumor and metastasis models remain limited by incomplete immune components, insufficient patient heterogeneity, and inadequate modeling of tumor-induced bone remodeling.[157,160,168] Infection-related models face additional challenges in reproducing host-pathogen interactions, immune activation, persistent intracellular infection, and drug-response testing.[103,106] Patient-specific models remain affected by donor variability, inconsistent differentiation, high cost, and limited clinical validation.[140,169]

Overall, these limitations indicate that the value of organoid-on-chip platforms depends on matching each model to a specific biological or translational question, validating it with appropriate functional readouts, and interpreting results within clearly defined technical and physiological boundaries.

7. Future perspectives

The convergence of bone organoids and microphysiological chip platforms is reshaping orthopedic research by enabling human-derived bone-like tissues to be maintained *in vitro* and guided toward maturation, pathological transformation, and therapeutic response under defined conditions.[17,170] Advances in 3D bioprinting, self-organization-based tissue formation, and microfluidic engineering have together provided increasingly practical routes to reconstruct multiscale structure–function relationships while improving standardization and experimental reproducibility.[14]

From a translational perspective, the near-term value of these systems is likely to lie primarily in preclinical and patient-specific applications rather than direct therapeutic implantation. Bone organoids and bone-on-chip models can support drug screening, mechanism-informed efficacy evaluation, toxicity assessment, and longitudinal monitoring of remodeling responses within human-relevant multicellular microenvironments.[11,139,169] In particular, patient-specific constructs derived from induced pluripotent stem cells may offer a feasible route for individualized testing of treatment sensitivity and optimization of therapeutic selection.[139,140,169]

Looking further ahead, these platforms may also support regenerative applications beyond *in vitro* modeling. Early preclinical studies suggest that engineered bone-like tissues can survive, integrate, and function *in vivo*, raising the possibility that living graft-like products derived from patient cells could eventually complement or replace conventional inert graft materials in complex bone repair.[126,171] Such a strategy would be especially attractive for large defects, where structural support, vascularization, and active participation in regeneration are all required.

Clinical translation and regulatory barriers should also be considered more explicitly. In the near term, 3D-bioprinted bone organoids and bone-on-chip systems are more likely to be translated into preclinical testing and patient-specific screening platforms than into implantable products.[134,169] Broader clinical adoption will require scalable and reproducible fabrication, Good Manufacturing Practice (GMP)-compatible workflows, clinically compatible materials, aseptic processing, and robust batch-to-batch quality control.[108,134,169] Cost is

another major barrier, particularly for patient-specific models that require individualized cell expansion, bioink preparation, chip fabrication, long-term culture, and multiparametric validation.[134,169] Compatibility with higher-throughput drug screening will require parallelized chip formats, automated handling, miniaturized readouts, and standardized data acquisition and analysis.[109,136,138] In polydimethylsiloxane-based chips, absorption of hydrophobic compounds may alter effective drug concentrations and confound pharmacological interpretation; therefore, material selection, surface modification, dosing validation, and appropriate material controls should be considered.[67] Sterilization and aseptic processing remain important challenges because the biological components of living constructs may be incompatible with conventional terminal sterilization procedures.[108] For regenerative applications, potential safety concerns include immunogenicity, tumorigenicity, ectopic ossification, uncontrolled differentiation, material degradation products, and long-term *in vivo* instability.[126,140,169] Regulatory translation will require clearly defined contexts of use, platform qualification, standardized performance and release criteria, traceability, and clinically relevant safety and efficacy endpoints.[108,134,169]

To provide a clearer roadmap for future adoption, the development of orthopedic organoid-on-chip systems can be viewed in short-, mid-, and long-term stages. In the short term, priority should be given to standardized fabrication protocols, shared validation criteria, reproducible readouts, and cross-study benchmarking. In the midterm, the field should focus on disease-specific and patient-specific platforms that integrate vascular, immune, marrow, endocrine, and mechanical modules, together with higher-throughput screening, biosensing, and longitudinal monitoring. In the long term, broader adoption will require regulatory qualification, GMP-compliant manufacturing, clinical outcome correlation, multi-organ integration, and careful evaluation of regenerative applications.[108,134,169] Overall, continued integration of stem cell biology, biofabrication, microphysiological engineering, and translational validation will be essential to move these systems from promising experimental models toward more reliable orthopedic research and clinical tools.

8. Conclusion

In conclusion, 3D-printed organoids and organoid-on-chip technologies provide an increasingly coherent engineering and biological foundation for investigating orthopedic diseases and evaluating therapeutic strategies. Despite persistent challenges in maturation, multicellular complexity, and standardization, these platforms are advancing orthopedic disease modeling, drug evaluation, and future regenerative strategies.

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Conflict of interest

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