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Modular construction of osteochondral organoids: Recapitulating gradients and microenvironment

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Abstract

In recent years, bone and cartilage organoid technology has attracted increasing attention and undergone rapid development, offering emerging approaches and broad prospects for osteoarthritis (OA) treatment, tissue engineering, regenerative medicine, and related fields. Despite the documented progress of bone and cartilage organoid models in structural biomimetic design, their limitation in the holistic reproduction of osteochondral unit, including interface gradient and physiological functions, remains a challenge for clinical application. Based on cutting-edge research, this review summarizes modular construction strategies of bone and cartilage organoids, targeting three types of signals in the osteochondral unit microenvironment: mechanical, metabolic, and immune. First, this review analyzes the microenvironment characteristics of the osteochondral unit, including the interface gradient and major signal pathways, then discusses the essential requirements for construction of osteochondral organoid. Then, based on different emphases, various osteochondral or cartilage organoid examples are classified and reviewed. Furthermore, this paper analyzes their advantages, bottlenecks, and feasibility, thereby outlining a design framework for modular organoids. Next, the technical pathways for functional coupling between modules are discussed, mainly including 3D bioprinting, organoid assembly, and microfluidic chips, in an attempt to provide solutions to the integrated challenge of osteochondral organoids. Then, the monitoring of multidimensional signals in organoids culture system is discussed. Finally, feasibility of artificial intelligence (AI) and machine learning in this field is discussed, with the aim to establish a closed-loop system for intelligent organoid culture.

Keywords: Organoid; Osteochondral; Osteoarthritis; Microenvironment; Artificial intelligence; 3D bioprinting

1. Introduction

As one of the most prevalent degenerative joint diseases, osteoarthritis (OA) imposes heavy public health and massive symptomatic burdens worldwide, with its prevalence remaining persistently high in recent years.[1] Accumulating evidence reveals that the core pathology of OA rests on the progressive functional collapse of the osteochondral unit, far beyond just structural degeneration of articular cartilage.[2] The osteochondral unit functions as an interfacial structure with a hierarchical gradient, comprising articular cartilage, calcified cartilage zone,

tidemark, and subchondral bone.[3] These soft and hard tissues constitute a coupled system capable of processing multiple external signals, enabling this system to continuously sense, decode, and respond to mechanical cues, metabolic progression, and immune factors, thereby sustaining joint homeostasis and physiological capacities.[2,3]

Recent studies have revealed that a core process of OA lies in the pathological advancement of the tidemark, leading to disruption of the soft-hard tissue protective barrier and crosstalk of multiple signals subsequently.[4] For example, proangiogenic factors and nerve growth factors first infiltrate the cartilage zone, acting synergistically and then leading to abnormal vascular ingrowth and excessive sympathetic nerve sprouting.[5] Then, inflammatory factors diffuse through vessels to the cartilage region, while cartilage becomes thinner and causes abnormal mechanical stress load. These pathological changes jointly stimulate the reprogramming of gene expression, leading to cartilage degeneration.[6,7] On the other hand, the initial subchondral bone injury also serves as an initiating factor of OA, causing bone remodeling uncoupling and leading to early bone resorption and late trabecular sclerosis.[8] Such factors mentioned above further disrupt mechanical signals and create a vicious cycle. Regenerative therapy for OA based on the disease pathogenesis and progression is needed to rebuild the signal insulation barrier and block the pathological process, thereby maintaining the homeostasis of the signal network.[9]

Table 1 shows key signaling pathways and their outcomes results in the context of OA. These pathways could potentially act as the programmable signals in modular construction of organoids to mimic the OA pathology.

Table 1. Key signaling pathways and implications for organoid design in osteoarthritis

Key pathway	Typical outcome	Potential application in organoid design	Ref.
Wnt/β-catenin	Excessive bone remodeling, degradation of bone structure	Modulating bone remodeling balance *in vitro*	10
YAP/TAZ	Increased expression of matrix-degrading enzymes, cartilage degeneration	Programming mechano-matrix homeostasis effectively	11
TGF-β3/Smad	Abnormal bone remodeling, subchondral bone sclerosis, degradation of cartilage matrix	Inducing chondrogenesis and matrix deposition	12
Integrin	Disruption of the intracellular anabolic–catabolic balance	Enhancing extracellular matrix anchoring and structural integrity	13
AMPK/mTOR	Chondrocyte apoptosis, cartilage degradation	Regulating metabolism to curb apoptosis	14,15
HIF-2α-NAMPT	Cartilage destruction, synovitis	Simulating hypoxia to protect cartilage	16
NF-κb	Increased expression of matrix-degrading enzymes, abnormally enhanced catabolism	Modulating inflammatory microenvironment and catabolism	17
JAK/STAT	Intense inflammatory response, cartilage degradation, programmed cell death	Controlling immune-inflammatory crosstalk precisely	14
MAPK	Cartilage breakdown, hypertrophy, cell death, secretion of pain mediators.	Suppressing hypertrophy and pain signals	14

Driven by the goals mentioned above, osteochondral organoid technology has become one of the main directions explored in the field. Organoids are micro-organ models constructed *in vitro*, which use the self-organization principle of stem cells.[18] Organoid models offer unique advantages in the direction of osteochondral repair and regeneration, thereby holding considerable research potential. Relying on various biomanufacturing and engineering technologies, existing research has constructed a series of biomimetic models of bone and cartilage *in vitro*, achieving a certain degree of spatial stratification and partial gradients.[19,20] Though bone and cartilage organoid technology has yielded certain results in the structural biomimetic paradigm, models often face challenges and bottlenecks such as angiogenesis and immunomodulation, especially during implantation *in vivo* and *in vitro* simulation.[21] In fact, most existing studies are limited to achieving gradient construction through structural bionics,

which present insufficient capacity to reconstruct multiple signal types *in vivo*. These models lack a systematic replication framework for the multiple signal network microenvironment, causing difficulties in accurately simulating the ability to perceive, integrate, and respond to multiple dynamic signals in the natural interface. The crux of such problems lies in the disconnection between static models and the dynamic processes of physiological development in real bone or cartilage units as well as the OA pathological microenvironment. The insufficient capability of interface signal processing makes it difficult for organoid models to accurately simulate real growth and development processes, and the dynamic pathological microenvironment. In real-word settings, a range of models present various deficiencies and fail to achieve long-term stable functional regeneration.

To address the above bottlenecks, this review proposes a systematic design framework for organoid culture for the gradient signal programming of osteochondral units, aiming to prompt the paradigm innovation of this field from static structure simulation to precise functional regeneration (Figure 1). This article seeks to utilize approaches such as materials science and engineering technology to design dynamic signal-programmable bone or cartilage organoid models. These models act as integrated components of integrated osteochondral organoids, and further employ engineering integration technology to achieve integrated osteochondral organoids. This article first reviews the structural gradient and microenvironment of the osteochondral unit. Then, based on existing research instances of bone and cartilage organoids, we systematically elaborate a module design framework oriented to signal processing function. This design includes how to perform functional programming of three important signals, namely mechanical, metabolic, and immune signals, aiming to achieve dynamic perception, integration, and response. Then, we explore how to assemble the functional modules of bone or cartilage organoids relying on cutting-edge technologies, with the intent to achieve structural and functional integration. Finally, this article introduces existing technologies of signal detection and processing, aiming to provide auxiliary analysis for organoid culture platforms. Further, we envision the technological transformation of next-generation organoids empowered by AI. Collectively, we present a specific modular design framework comprising five steps that renders the principles of osteochondral organoid construction more accessible and

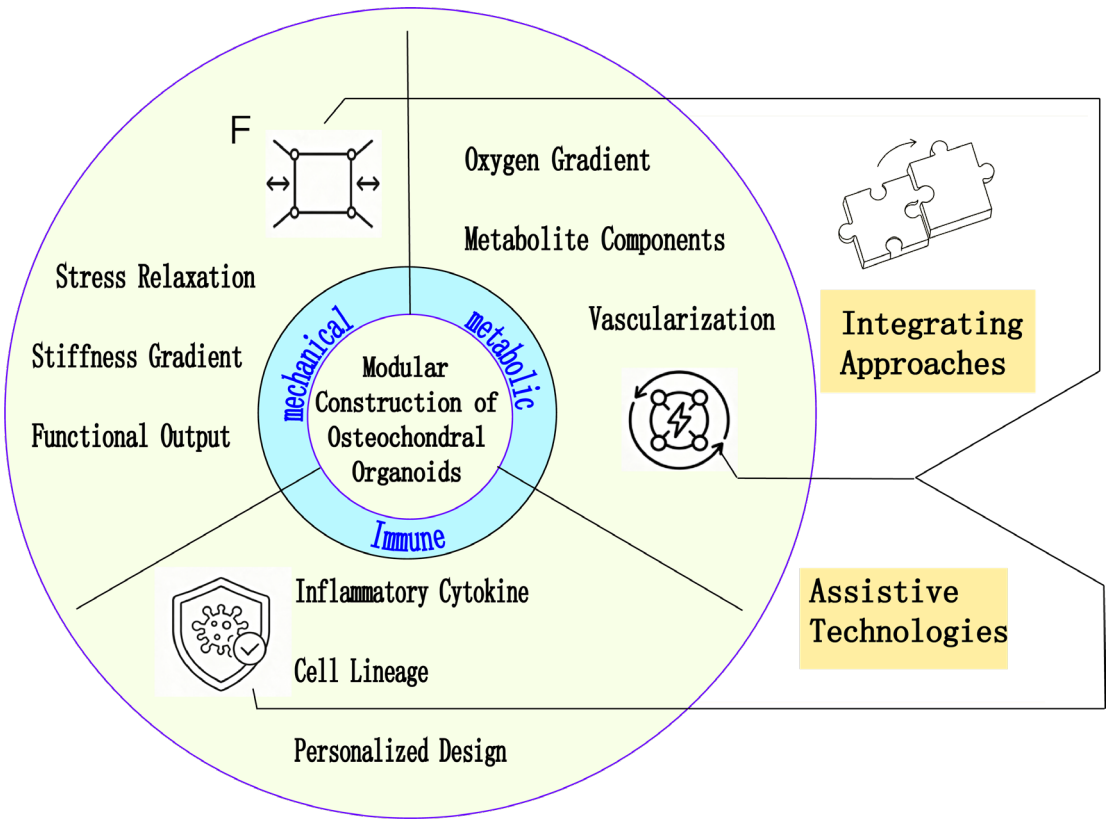


Figure 1. Modular design of integrated osteochondral organoids. This framework mainly involves three modules: mechanical, metabolic, and immune. Further, these modules could be tightly coupled by integrating approaches and assistive technologies. Moreover, other modules have the potential to be coupled together to recapitulate the osteochondral microenvironment.

actionable: spatial zoning, mechanical module, metabolic module, immune module, and coupling validation

2. Microenvironment in the osteochondral unit

2.1. Cartilage microenvironment

The mechanical microenvironment is a core feature of cartilage, and hydrostatic pressure is gradually recognized as one of the most important stimuli.[22] This mechanical stimulus is crucial for the maintenance of chondrocyte phenotype and matrix synthesis. Mechanical compression loading of appropriate intensity and frequency will contribute to matrix synthesis and accumulation of chondrocytes, mainly involving the synthesis of type II collagen and proteoglycans,[23] whereas excessive mechanical loading or abnormal shear stress tends to trigger degenerative death of chondrocytes and matrix degradation.[24] Chondrocytes sense and respond to external mechanical stimuli through multiple mechanoreceptors, including integrin, primary cilia, and ion channels. Through such pathways, these mechanosensitive cells convert such physical signals into intracellular biochemical signals.[25,26] Compared to most other tissues in humans, a hypoxic environment is one of the most prominent features of cartilage, with a low oxygen tension from 1% to 4% within the tissue.[27] Articular cartilage is an avascular tissue, meaning the transportation of nutrients and oxygen always depends on the diffusion of synovial fluid. This mechanism leads to a hypoxic environment and low nutrient supply in the cartilage zone. This hypoxic environment plays an important role in the differentiation, maturation, and phenotype maintenance of cells in the cartilage zone. This process typically involves high expression of genes regulated by hypoxia-inducible factor HIF-1α under low oxygen tension.[28] As the main energy source of chondrocytes, the glucose concentration in synovial fluid tends to be at a low level. Meanwhile, the metabolic mechanism of chondrocytes is mainly glycolysis rather than oxidative phosphorylation, which induces limited energy supply in chondrocytes.[29]

Based on morphological observation, owing to the absence of blood vessels and the dense extracellular matrix (ECM), articular cartilage typically possesses tissue immunological privilege. In the cartilage region, chondrocytes maintain immune homeostasis mainly through the synergistic action of the physical barrier, involving the matrix and immunomodulatory factors such as transforming growth factor beta (TGF-β).[30] In OA, the cartilage microenvironment manifests as the disruption of this immunological privilege. In this process, pro-inflammatory factors, such as interleukin (IL)-1α and tumor necrosis factor alpha (TNF-α), lead to cartilage matrix degradation and metabolic imbalance, which further exacerbate the lesions through positive feedback loops.[31]

2.2 Bone microenvironment

Compared with cartilage, bone tissue exhibits an internal architecture with high vascularization. Among these vessels, type L vessels act as the major structural component, responsible for hematopoiesis in the bone marrow and substance circulation. In addition, type L vessels mainly ensure the delivery of nutrients and growth factors and the maintenance of the oxygen tension gradient within the bone region.[32] Besides, type H vessels function as the characteristic vessels of osteogenesis. Through tight coupling with bone, type H vessels play a key role in bone growth and development, steady-state maintenance, and remodeling balance. Type H vessels induce bone formation through several signaling pathways, including platelet-derived growth factor-BB (PDGF-BB), hypoxia-inducible factor 1-alpha (HIF-1α), and vascular endothelial growth factor (VEGF). These pathways jointly mediate the dynamic coupling of angiogenesis and osteogenesis.[33] The bone remodeling process encompasses the attachment and resorption of osteoclasts and the migration, secretion, and mineralization of osteoblasts. This process primarily involves the resorption-formation coupling of hydroxyapatite and collagen, thereby maintaining and renewing the structural integrity of bone tissue.[34]

The ECM of bone tissue is rich in type I collagen and hydroxyapatite. These components endow bone with a high stiffness, two to three orders of magnitude higher than that of cartilage. High stiffness enables bone tissue to withstand multimodal mechanical stimuli under physiological conditions, such as compression, stretching, and fluid shear stress.[35] Conversely, these mechanical stimuli serve as external signals to regulate bone mass and structural formation through negative feedback mechanisms. This process utilizes the mechanical load-sensing network formed by the cellular composition of bones, mainly involving integrin, Wnt/β-catenin, and YAP-TAZ pathways. Mechanical stimulus exhibits a dual-faced dynamic adaptation, causing the promotion of bone formation induced by mechanical loading and the suppression of bone formation induced by mechanical unloading.[10,11,13]

2.3. Tidemark region and gradient characteristics

The tidemark functions as the boundary between articular hyaline cartilage and calcified cartilage, reflecting the transitional gradient characteristics of the osteochondral unit. Across this transitional boundary zone, from articular cartilage and calcified cartilage to subchondral bone, the elastic modulus of the osteochondral unit features a stiffness increase of two to three orders of magnitude.[36] Moreover, the tidemark blocks vascular ingrowth, cell

migration, and cytokine diffusion. This barrier contributes to maintain the immune privilege of the cartilage region.[30] Tidemark advancement is also regarded as a key feature of OA pathology. This pathological process often marks the disruption of the barrier function of the tidemark, triggering abnormal ingrowth vessel and nerve, along with inflammatory factor invasion, which finally result in signal crosstalk and cartilage degeneration.[4]

Exemplified by the tidemark, the osteochondral unit exhibits interfacial features of multiple gradient and integral coupling in both structure and function. The continuous transition of cell phenotypes and matrix components forms its mechanical gradient, ensuring smooth bearing and buffering against external mechanical stimuli and loading.[36] In addition, cartilage secretion transmits downstream signals to regulate bone remodeling, while the upstream pathway of bone metabolism maintains cartilage nutrition delivery and matrix homeostasis. This bidirectional regulation forms a closed coupling loop, performing a significant role in signal communication between cartilage and bone.

Construction strategy for cartilage organoids utilized in existing research are relatively mature (Figure 2). Nevertheless, several challenges continue to limit the application of bone organoids, including inadequate mechanical stiffness, insufficient vascularization, and poor batch-to-batch reproducibility. At present, cutting-edge research trends gradually shift from biomimetic construction toward integrative, unified construction of osteochondral unit. The primary goal is to recapitulate the microenvironmental and signaling interaction pathways of the interface to achieve synergistic development of osteochondral organoids and high-fidelity simulation of their structural and functional gradients.[20] During the construction of integrated osteochondral organoids, the preservation of possible signal interactions in physiological processes is expected to help overcome existing technical bottlenecks such as vascularization and batch repeatability.

Given that the direct constructing techniques for integrated osteochondral organoids remains immature, this article adopts an anatomy-reconstruction approach for modular design based on existing technical foundations. In addition, we analyze the microenvironment and typical signal types in the real physiological processes of the osteochondral unit, encompassing mechanical, metabolic, and immune modules. Furthermore, we discuss several integrated technologies that have the potential to be used in module recombination, including organoid chips and 3D bioprinting. Finally, the review ends with a prospective discussion on intelligent, closed-loop design based on machine learning.

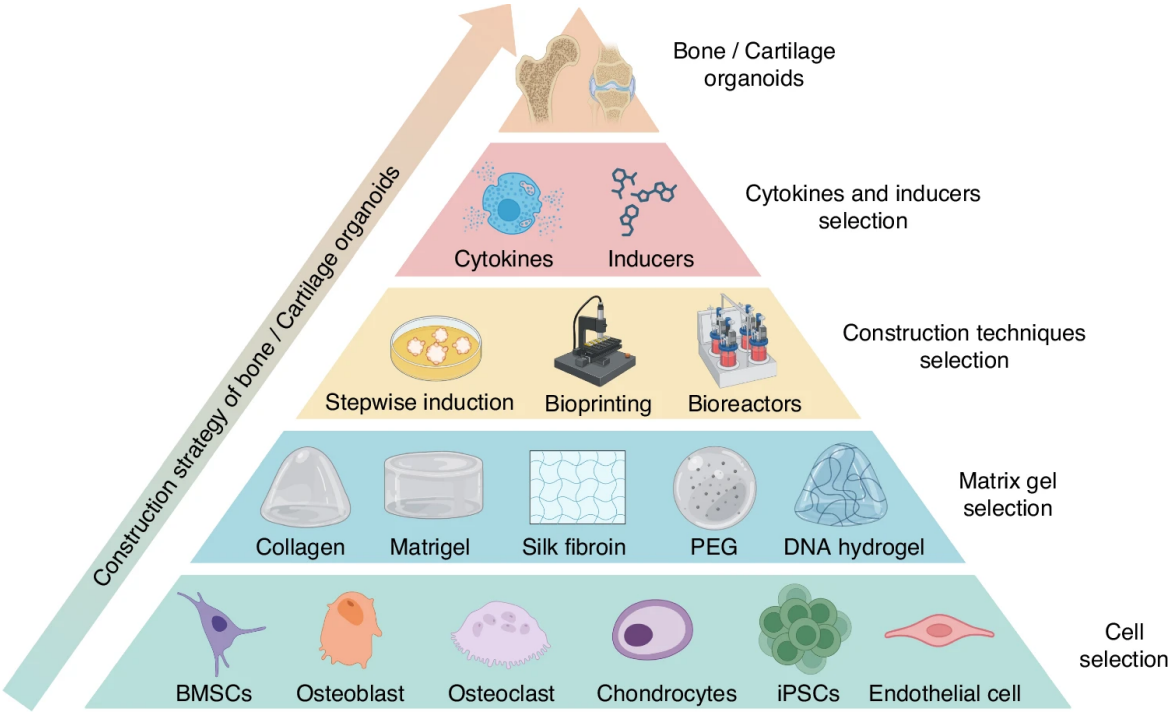


Figure 2. Major construction strategies for bone or cartilage organoids. This construction process of bone/cartilage organoids requires the gradual and progressive selection of cells, matrix gel, construction techniques, cytokines, and inducers.[20]

3. Mechanical module

The cellular microenvironment within the ECM is one of the key factors determining cell fate.[37] According to cutting-edge studies, in the mechanical microenvironment of three-dimensional cell and organoid cultures, the mechanical support and stress relaxation of viscoelastic matrices are critically important.[38] Excellent stress relaxation properties can promote chondrocyte proliferation and inhibit cell death. Therefore, this mechanical property facilitates the deposition and formation of the cartilage matrix and supports superior self-organization behavior.[39] The framework of the mechanical signaling module is expected to be designed on the basis of an osteochondral gradient structure with dynamic internal connectivity. Further, this module incorporates biomimetic matrix materials with stress relaxation properties, aiming to promote self-organized proliferation and differentiation of cells. Then, this will accelerate their subsequent self-organization and evolution into functionally mature organoids.

3.1. Mechanical gradient

The essence of the osteochondral unit is a continuous mechanical gradient field spanning from cartilage to bone, with mechanical strength spanning two to three orders of magnitude (from MPa to GPa). This construction functions to minimize stress concentration to the greatest extent and then guides specific cells to differentiate at specific locations. This process facilitates the construction of tissue interfaces with complex functions from the bottom up.[36,40] The design basis of the mechanical encoding module is to establish a biomimetic structure with a continuous mechanical gradient. This mechanical gradient would contribute to the reproduction of cell behavior building upon static model construction.

The biomimetic gradient-structured cartilage organoids designed by Miao *et al.*[41] utilize droplet microfluidics technology and dCSEV hydrogel to form a complete structure with heterogeneous microcartilage. Meanwhile, the dCSEV hydrogel maintains the specific protein secretion capacity of chondrocytes. The 3D dCSEV reprogramming system is used to promote cell rejuvenation through signals such as SOX9, thus achieving long-term stability of organoid function. Furthermore, Miao *et al.* applied periodic mechanical stimulation to the constructed OA chip model to simulate the pathological microenvironment. These results demonstrated that this hydrogel sustains the release of protective factors. The spatial distribution of mechanical gradients forms the basis for the mechanical design of organoids.[41] Beyond static biomimetic structures and single mechanical stimuli, it remains necessary to further simulate the OA pathological microenvironment and physiological processes, such as focal cartilage softening, nerve invasion, and closed-loop progressive simulation of OA.

3.2. Stress relaxation

Stress relaxation parameters can be dynamically modulated through material design strategies such as supramolecular and complex crosslinking. This design mainly aims for temporal programmability of mechanical signals.[42] Yang and colleagues[43] utilized host-guest complexes to prepare supramolecular HA-TP hydrogels, realizing rapid stress relaxation with a half stress relaxation time of 9.7 s. In addition, its highly dynamic network structure exhibited satisfactory adaptability to volumetric expansion during cartilage organoid formation. Moreover, the hydrogel materials designed by Anseth's team also provided some inspiration. By modulating covalently adaptable networks, they formed an interconnected articular cartilage-specific matrix in formulation experiments with an average relaxation time of 3 days ($2.\ 6 \times 10^5$ s). This material also reached high cell density and accurate matrix deposition.[42] The aforementioned studies support the feasibility of precise encoding to realize stress relaxation through materials design. Further, dynamically adaptable network structures responsive to mechanical signals can be constructed to prepare viscoelastic matrices with excellent mechanical properties and topological structures. Notably, the stress relaxation behavior of native osteochondral tissue exhibits nonlinear and heterogeneous characteristics.[44] Therefore, a single temporal relaxation parameter design might be challenging to capture the physiological complexity of the osteochondral. This aspect necessitates further multiscale and multimodal mechanical programming.

3.3. Functional output

Mechanical module programming ultimately directs the regulation of cell fate through the spatiotemporal control of gradient structure and viscoelastic parameters via a series of signaling cascade networks. These signals activate downstream pathways (for example, YAP/TAZ) through sensors such as integrins and determine chondrogenic or osteogenic differentiation and regulate ECM synthesis. A series of pathways ultimately points to the overall functional output from cells to tissues.[45]

Su's team used microfluidic technology to develop RGD-modified silk fibroin-DNA hydrogel microspheres for the construction of cartilage organoid precursors. The results showed that this hydrogel significantly enhanced chondrogenic differentiation of bone marrow mesenchymal stem cells through a series of mechanisms, including integrin-mediated adhesion pathways. Meanwhile, the regeneration of bone tissue was remarkably augmented.[46] Additionally, the dynamic GelMA/DNA double-network hydrogel developed by this team is also notable. This design recapitulates the biological cues of moderate mechanical strength and excellent viscoelasticity of skeletal ECM, realizing uniform mineralization, interconnected cellular

networks, and favorable cell survival rates.[47] Through spatiotemporal modulation of the mechanical properties of the osteochondral unit, this team has designed biomaterials to recapitulate physiological functions. Collectively, the aforementioned designs demonstrate mechanical tunability, self-healing capability, and stimulus responsiveness, which may facilitate the repair of pathological conditions in OA. Existing challenges may lie in the precise design of material formulations and the complete structural recapitulation during osteochondral development, to meet the precision demands of regenerative medicine.

4. Metabolic signaling module

In the osteochondral unit, the metabolic gradient exhibits a zonal distribution, such as a hypoxic environment in the superficial cartilage and a normoxic environment in the deep bone. Meanwhile, the spatiotemporal distribution of specific metabolites plays a decisive role in regulating cell fate and differential function, such as lactate.[48,49] The design of the metabolic module mainly involves three components:oxygen gradients, metabolites, and vascularization. The oxygen gradient design aims to maintain the spatial heterogeneity basis of organoids. On this basis, programmed specific metabolites are further utilized as diverse signaling factors to actively regulate cell fate and functional maturation. In addition, these signals serve as a bridge connecting mechanical stimulation and immune function. Furthermore, solutions are devised for vascularization to meet the requirements of intelligent organoid system integration.

4.1. Maintenance of oxygen gradients.

The oxygen gradient represents the most fundamental and important feature of metabolic gradient in the osteochondral unit. Spatial differences in oxygen tension determine cell phenotype differentiation to a large extent. The oxygen gradient mainly encompasses the hypoxic zone of articular cartilage (approximately 1% to 4%) and the normoxic environment of subchondral bone (approximately 5% to 10%).[36,50] Oxygen gradients are primarily established through active regulation from the external environment and internal material programming. Thus, it could be more optional and controllable to construct stable oxygen concentration gradients within 3D space and then enable active microenvironmental regulation internally.

Yang *et al.*[43] designed a supramolecular hydrogel capable of accommodating the volumetric expansion of encapsulated mesenchymal stromal cells (MSCs) while spontaneously promoting the establishment of a hypoxic environment. This hydrogel facilitates the formation of large multicellular cartilage organoids, displaying broad universality. Deng *et al.*[51] developed a bone organoid unit based on silk fibroin hydrogel microspheres. The introduction of an internal calcium peroxide-HAP oxygen-releasing system and immune cells enabled the formation of a bone organoid unit with high cell viability after 28 days of culture. These two studies support the feasibility of internal and external oxygen gradients and microenvironmental programming, holding promise to provide personalized regeneration strategies for large-area bone defects. Notably, the hydrogels designed by Yang *et al.* and Deng *et al.* regulate oxygen gradients both through endogenous mechanisms, involving guidance and release. Future trends may shift toward oxygen gradient-responsive intelligent materials. This underscores a great need for spontaneous performance in dynamic oxygen gradient adjustments according to developmental stages or pathological conditions, thereby fulfilling closed-loop precise simulation.

4.2. Metabolic signal programming

The oxygen gradient within the osteochondral unit determines differential metabolic activities, while metabolites such as lactate and reactive oxygen species serve as molecules for epigenetic regulation and signal transduction. These signals precisely regulate the lineage specification and functional execution of chondrocytes and osteoblasts.[52,53] Based on this principle, precise programming of parameters such as metabolic type and metabolite concentration in this section is essential. It is expected to enable the predetermined control of developmental progression and functional output of organoids.

Wang's team developed an anti-aging cartilage organoid scaffold using 3D bioprinting technology, targeting metabolic reprogramming through the miR-23b/ELOVL5 axis. This manipulation achieved simultaneous regulation of glycolysis and fatty acid oxidation, effectively promoting cartilage repair and delaying cellular senescence.[54] Yang *et al.*[43] designed a supramolecular hydrogel that creates a hypoxic environment to drive glycolytic reprogramming. Through the utilization of the metabolite lactate to directly catalyze histone H3K18 lactylation modification, they successfully regulated epigenetic instructions and drove ECM synthesis and deposition. The aforementioned studies demonstrate the decisive role of metabolic axes and products in regulating cell fate and function, and further achieve specific physiological behaviors through targeted design in practice. Encoding specific signals during organoid construction holds promise for precise regeneration of structure and function. The current challenge lies in the requirements for synergistic control of multiple metabolic signals. Developing strategies for networked signal programming could enable more precise and efficient regulation of these interconnected signals, particularly within the complex physiological microenvironment *in vivo*.

4.3. Vascularization strategies

The integration of vascularized functional networks currently exists as one of the greatest challenges facing organoid technology.[55] The vascular network is an essential approach for constructing organoids at millimeter-scale dimensions or larger, responsible for the transport of oxygen, nutrients, and metabolic waste. Notably, vascularization also plays a significant role in supporting long-term survival rates and microenvironmental homeostasis.[56,57] In the metabolic module, vascularization serves as a key bridge for microenvironmental recapitulation and multifunctional coupling systems. Further, it holds significant importance in the developmental physiological processes of organoids and OA disease modeling.

The dual-chamber microfluidic chip designed by Salehi *et al.*[58] utilized multicellular co-culture to construct self-assembled vascular networks. They successfully modeled a bone–blood vessel coupling system, and further employed IL-1β to mimic the OA pathological microenvironment for anti-inflammatory drug testing. Kesharwani *et al.*[59] used ESC-derived sclerotome cells mixed with EGFP-labeled HUVECs to achieve the construction of vascular networks at the early stage of cartilage ossification. Their subsequent research verified the perfusion capacity of the vascular-like network. These two studies using cell co-culture and microfluidic chips present two vascularization construction strategies: internal pre-patterning and external guidance. The former facilitates *in situ* angiogenesis and bone-vascular tissue coupling, hence enabling disease modeling and drug screening. The latter recapitulates the physiological process of cartilage ossification and provides engineering strategies for vascular network chips. Future directions consist of enhancing the complexity and stability of vascular networks and reaching multi-gradient coupling with metabolic and mechanical signals. Through these strategies, it is expected to construct biomimetic organoid models with mature functional gradients.

5. Immune signaling module

The typical features of core pathological processes in OA mainly include inflammatory cytokine storms and immune cell aggregation.[60] The primary task of this immune module is to recapitulate these processes dynamically and then provide organoids with relatively mature capacity to modulate the inflammatory microenvironment. The aim of this module lies in the establishment of standardized and personalized disease models as well as drug screening. This module will be designed to use pro-inflammatory cytokines to simulate the degenerative progression of joint arthritis, while employing techniques such as multicellular co-culture to recapitulate the immune microenvironment. Thus, the construction of a highly biomimetic pathological microenvironment is facilitated through the precise regulation of both inflammatory cytokines and immune cells. Furthermore, patient-derived autologous cells can be incorporated to achieve personalized precision medicine and promote high-value clinical translation.

5.1. Inflammatory cytokines programming

In OA, the immune cells and chondrocytes recruited by the joint cavity continuously release proinflammatory cytokines, such as IL-1β and TNF-α. These proinflammatory signals activate downstream inflammatory networks and subsequently lead to pathological changes such as cartilage degeneration and bone remodeling.[61] Based on this principle, direct external input of inflammatory cytokines as a stimulus source is expected to facilitate rapid construction of organoid models with OA pathological features.

Dönges's team designed a culture medium containing prohypertrophic factors and inflammatory cytokines, including IL-6, IL-1β and TNF-α, to simulate the effects of inflammatory synovial fluid. They then used cultured hBMSCs to derive OA cartilage organoids. The results demonstrated that these organoids exhibited molecular, cellular and compositional characteristics similar to the degenerative and catabolic inflammatory features of osteoarthritic cartilage. Moreover, subsequent experiments confirmed the effectiveness of this model in pathway testing and drug screening.[62] This study showed the feasibility of constructing pathological microenvironments in organoids induced by inflammatory cytokine programming. In addition, the notable advantages of this strategy include its simplicity, efficiency, and reproducibility. These capacities are conducive to large-scale drug screening, particularly for testing small-molecule inhibitors of inflammatory pathways. However, the limitations of this method cannot be ignored. The strategy remains insufficient to simulate the cytotoxic effects of immune cells or immune behaviors such as antigen-specific responses. Thus, it may have inherent bottlenecks in the selection of pathway types for drug screening.

5.2. Cell lineage encoding

Immune cell aggregation in OA includes endothelial cells, immune cells such as B and T cells, fibroblast-like cells, and macrophage-like cells. These immune changes can induce joint-specific and systemic low-grade inflammation.[60,63] Multicell co-culture, meaning that two or more functional cell types are cultured together in 3D space, provides a potential approach for recapitulating the immune microenvironment at the cellular level and holds promise for modeling this dynamic interaction network with controllability and accuracy.[64]

Gao *et al.*[65] established a 3D coculture system encapsulating synovial fibroblasts, endothelial cells,

and polarized macrophages. Then, they systematically analyzed the gene expression, factor secretion, and vascular networks under different macrophage phenotypes. Further, they reproduced the local inflammatory response induced by clinical biomaterial wear debris. This co-culture system incorporates three key effector cells involved in synovial inflammation, allowing this process to be more authentic and accurate. In addition, the combination of clinically oriented inquiry with multidimensional analysis demonstrated that the system is conducive to investigations across multiple research directions. Despite these outcomes, the requirements of different cell lineages for the growth environment remain large. Notably, disturbances such as the diffusion of paracrine factors and the degradation of raw materials often give rise to a series of problems, including signal crosstalk and changes in microenvironmental components. Considering the holistic dynamic interconnections and biochemical infiltration, complex multi-cell lineages have inherent conflicts in conditions and spatiotemporal dynamics. The bottlenecks and pain points in feasibility still remain as well.[66] This design can serve as a basic strategy through modifying cell formulation. Further, by integrating with other organoid construction techniques, this technology could achieve high-level biomimetic recapitulation of the immune microenvironment.[67]

5.3. Personalized design

Because of the enormous heterogeneity among patients, the personalized paradigm enhancement of immune signaling modules is indispensable. Moreover, it represents a necessary pathway for achieving practical clinical translation with large requirements. The principle of personalized precision medicine is based on patient-derived induced pluripotent stem cells (iPSC). Through directed differentiation to construct organoid models *in vitro* with matched genetic background and patient-specific pathology, this aspect is beneficial to promote the personalization of medical approaches such as drug intervention.[68,69]

Peng *et al.*[70] obtained an osteochondral interface model with cartilage repair capacity through spatial biological assembly of iPSC-derived iChon organoids and human periosteum-derived cell (hPDC) organoids. Conversely, Boone *et al.*[71] isolated chondrocytes from patients undergoing joint replacement surgery for OA to construct a human high-throughput aging cartilage organoid model. They established a highly personalized platform for simulating OA cartilage aging and high-throughput anti-aging compound screening. Currently, research on directly using patient-derived iPSC cells to reconstruct osteochondral organoids with patient-specific immune microenvironments remains relatively immature. Integrating the two aforementioned pathways could potentially help address this challenge. Nevertheless, this integrated strategy still has limitations in long-term dynamic monitoring. There remain opportunities to overcome this through immune cell co-culture and intelligent integrated approaches, with the aim of meeting higher demands of future personalized medicine.

6. Module coupling and technological routes

Through material composition design and other various technological approaches, the above studies have partially achieved functional recapitulation of the mechanical, metabolic, and immune modules of the osteochondral unit. Further, the results supported the feasibility and necessity of modular design. The future trend and challenge lie in the realization of cross-coupling of gradients. This acts as the key role in establishing communication bridges between modules and subsequently simulating the dynamic microenvironment of the multi-gradient signaling network in the osteochondral unit. Hence, organoid models can better recapitulate and optimize biological complexity, physiological fidelity, and interindividual variability in biomimetic functions. This section introduces several frontier coupling strategies for functional modules, including gradient 3D bioprinting, organoid assemblies, and organoids-on-chip. Table 2 summarizes the advantages and disadvantages of the three construction techniques. Given their distinct characteristics, the selection of an appropriate technique should be tailored to the specific requirements of organoid construction. Moreover, combining these techniques may help overcome their individual limitations and enhance the efficiency and versatility of organoid fabrication.

3D bioprinting is an additive manufacturing technique in which bioinks are constructed into structures with gradients *in vitro* (Figure 3). Major methods of 3D bioprinting cover coaxial printing, layer-by-layer deposition, photopolymerization, and so on. Numerous studies have demonstrated that 3D bioprinting offers significant advantages over traditional manufacturing techniques, including the formation of complex gradients, multiscale features, high precision, high resolution, and personalization. Mechanical properties are considered a notable advantage of 3D bioprinting technology. By adjusting printing parameters including porosity, layer thickness, and deposition direction, 3D bioprinting provides high accuracy to achieve a high degree of simulation of the continuous transition of the osteochondral unit.[72] The core to meet the predetermined functional requirements through 3D bioprinting lies in the design of bioink formulations. Bioinks are the basic building blocks of bioprinting, encapsulating the required cell types, growth factors, and biomaterials to construct

Table 2. Comparison of advantages and disadvantages of the three construction techniques

Techniques	Advantages	Disadvantages
3D bioprinting	Complex gradient formation; Multiscale feature fabrication; High precision and resolution	Limited biomimetic biochemical cues; Cell lineage specification challenges; Large-size construction difficulty
Organoid assembloids	Modular and flexible assembly; Recapitulate tissue complexity; Scalability and spatial integration	Limited mechanical load-bearing; Insufficient growth factors; Lacking microenvironment simulation
Organoid-on-chip	Flexible spatiotemporal modulation; Precise microenvironment regulation; Programmable input signals	Complexity of multi-chamber design; Fluid control challenges; Need of mechanical simulation

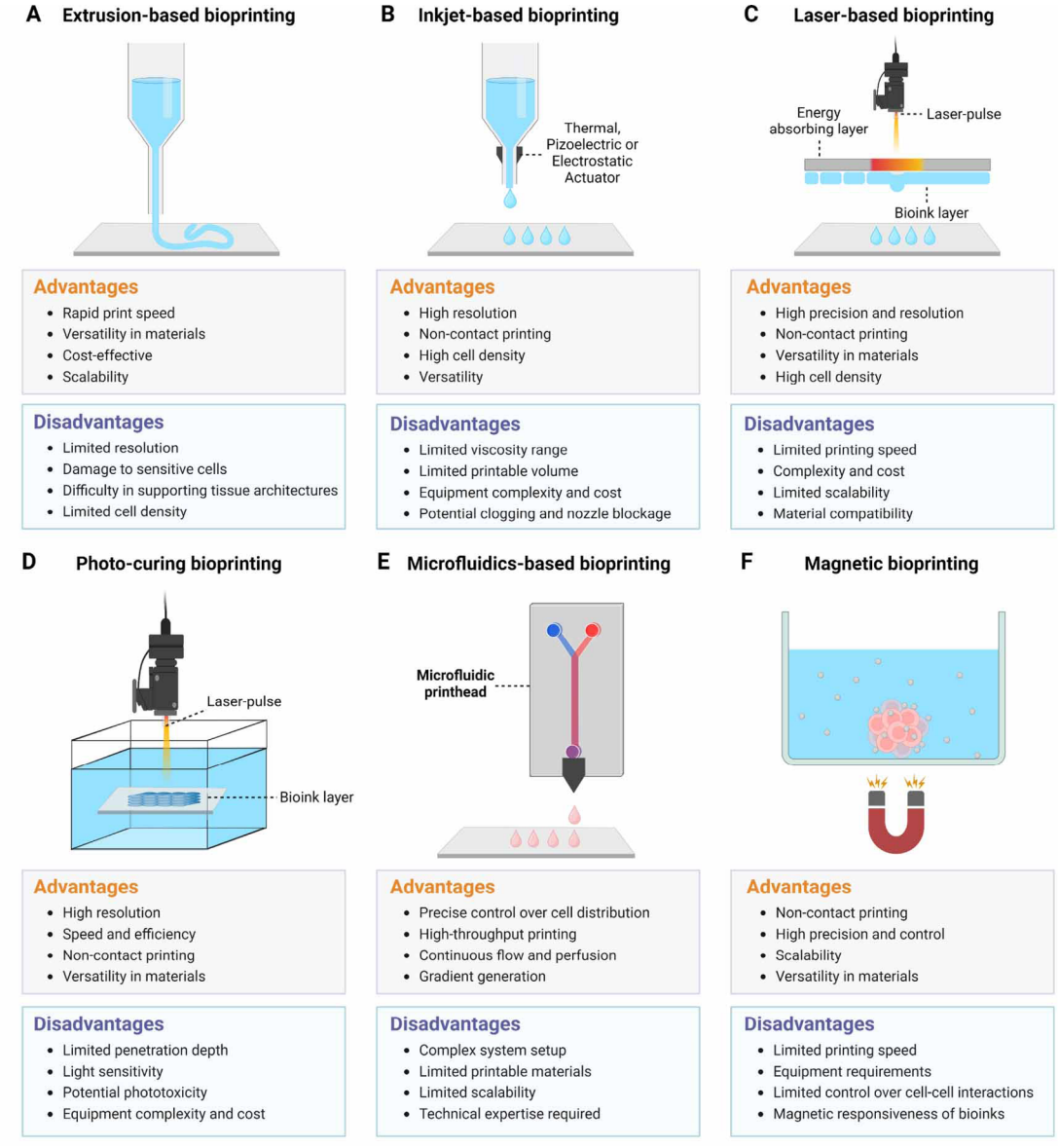


Figure 3. Commonly used 3D bioprinting techniques and their advantages and disadvantages. Appropriate selection is required according to these characteristics when constructing organoids.[73]

the functional biomimetic structures of organoids.[73] By means of multiple strategies, such as material modification and cytokine loading, 3D-printed organoid scaffolds can fulfill various functions. Major applications include multi-cell differentiation and phenotype induction, multilayer mechanical gradient construction, and controlled release of growth factors. Such advantages are suitable for *in vitro* modeling of osteochondral tissues or defect repair.[74,75] In the future, the frontier trend of 3D bioprinting technology depends on the further optimization of bioink formulation. This process covers multiple aspects, involving biocompatibility, rheological characteristics and functional integration. In addition, bottlenecks remain in further constructing complex continuous gradient structures, and biomimetic biochemical cues. Furthermore, challenges still exist in resolving interfacial stress gradients, cell lineage specification, and the construction of large-scale organoids.[76]

Organoid assembloids are formed by assembling single-type organoid models through bioadhesives, with the capacity to organize multiple cell types and organoids (Figure 4). Therefore, assembloid models can recapitulate tissue complexity and inter-tissue functional coordination.[77] Assembloids can precisely control the spatial distribution of cells and then mimic dynamic signal exchange and interactions within cells, between cell networks, and with the ECM. Notably, assembloids are equipped with significant advantages in addressing bottlenecks in bone organoid construction, including integration of the vascularization of endothelial cells and vascular cells, construction of immune microenvironment with a series of immune cells or components. In addition, the assembloid strategy can enhance nutrient diffusion and waste clearance, thus substantially promoting cell survival rate and functional stability in the long term.[78] For example, the NOVA system developed by Ju *et al.*,[79] combining bioprinting and bioadhesives, enables high-throughput modular living assembly of bone organoids. This system demonstrates favorable porosity, mechanical stability, adhesive properties, cell viability, and biocompatibility. Moreover, the NOVA system achieves internal bone-vascular coupling as well as an immune microenvironment with pro-regenerative functions. Another advantage of this strategy lies in its scalability and spatial integration capability. Owing to its compatibility with supporting the modular and flexible assembly of heterogeneous units, it is expected to enable more complex structural and functional coupling in the future and address the holistic biomimicry of osteochondral units.[80] In view of the major limitations in the mechanical load-bearing capacity of current bone organoids, it remains necessary to integrate mechanical stress stimulation, growth factor delivery, and other approaches to mimic the real mechanical microenvironment. Overall, assembloids provide a considerable chance to improve the physiological maturation and functional stability of bone organoids.[81]

Organ-on-chip refers to tissues or miniature organs cultured *in vitro* on microfluidic chips with multiple cell types and culture medium, commonly used to simulate and study the physiological behavior of organs and cells (Figure 5).[82,83] In recent years, progress has been gradually made in the technological convergence of organoids and organ-on-chip, attracting increasing attention in the field. Organoid-on-chip systems integrate the technical advantages of microfluidic chips and organoids, mainly including the precise regulation of the microenvironment, accurate simulation of multi-tissue interactions, and low heterogeneity. Osteochondral organoid-on-chip platforms are mainly composed of structural supports (substrate materials, hydrogels, and scaffolds), information monitoring components (biosensors), and cell sources (including a series of related cell types such as bone, cartilage, blood vessels, and nerve cells). In addition, organoid-on-chip systems can establish controllable conditions for disease modeling, which can be applied in bone metabolism, arthritis, and tumor metastasis.[84] A distinct strength of microfluidic technology exists in its dynamic perfusion characteristics, with the potential to address bottlenecks such as insufficient nutrient supply and lack of mechanical cues. Of course, this goal requires precise control of fluid movement and high-fidelity simulation of the tissue mechanical environment. Notably, owing to the multi-chamber characteristics of microfluidic chips, the ability to program the input signals can potentially be achieved by selecting specific culture media and factor components. This functions as a key bridge in enabling flexible spatiotemporal modulation of individual functional modules. In addition, cutting-edge studies have yielded certain results in the direction of the combination of microfluidics and machine learning. These two technologies may jointly facilitate the upgrade of organoid chips into intelligent and autonomous platforms. Through approaches such as Bayesian optimization and convolutional neural networks, machine learning can also drive real-time control of flow rates and manipulation of droplet size. In addition, these pathways can simultaneously conduct a series of functions such as cell sorting and cell counting.[85]

7. Assistive and intelligent technologies

The aforementioned parts of this article have elaborated on the basic logic and specific strategies for programming the mechanical, metabolic, and immune signals. Further, we introduce technologies that can be utilized in an integrated platform to provide resolutions and pathways for functional coupling among modules. The microenvironment within the integrated platform remains multidimensional and

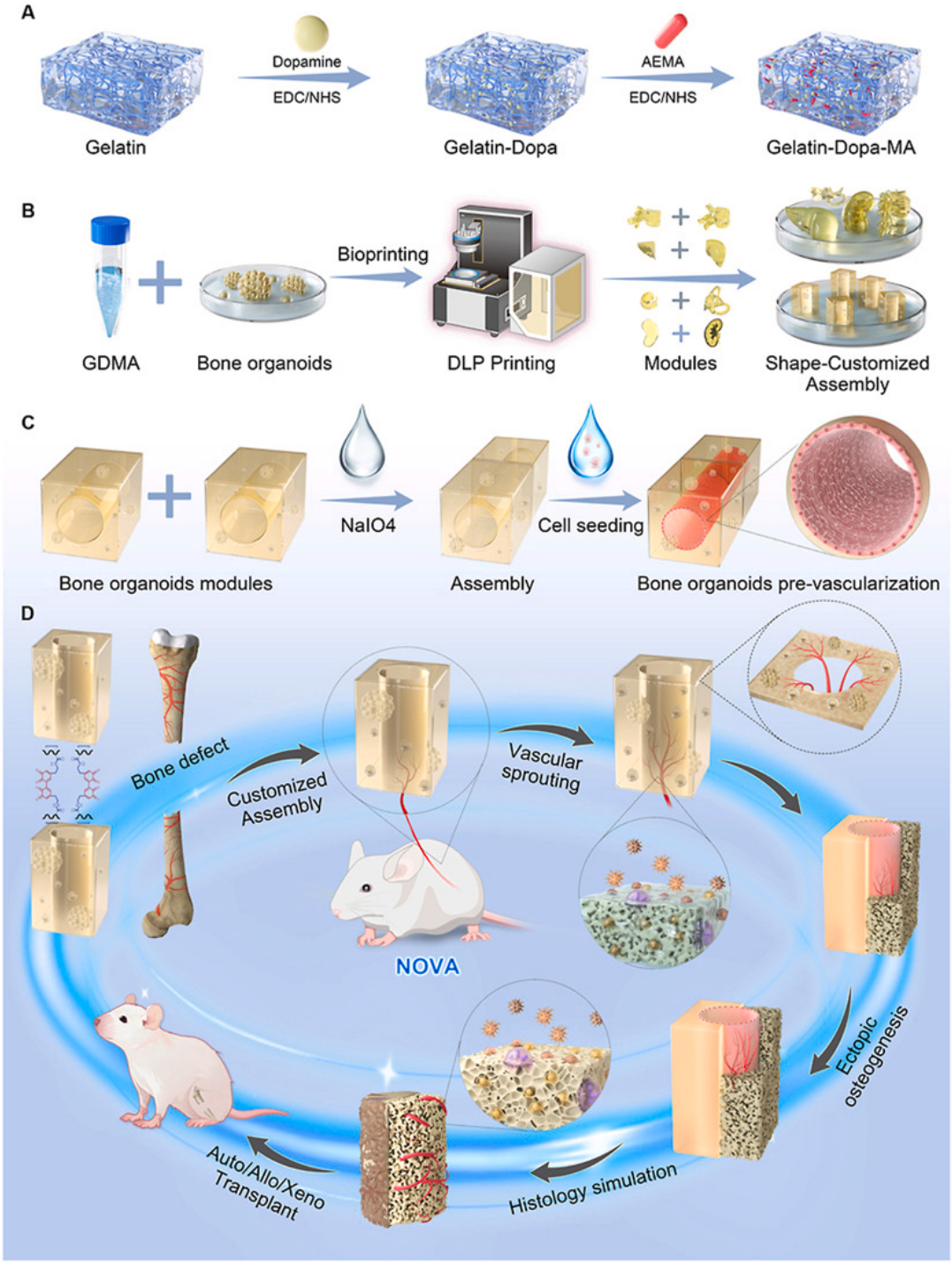


Figure 4. The framework of the NOVA system, an organoid assembloid exhibiting good vascular regeneration ability. This flowchart depicts the process from material preparation to structural assembly. Reproduced from Ju J, et al.79 Copyright © 2026 The Authors. Published by Elsevier B.V. on behalf of KeAi Communications Co. Ltd.

extremely complex. Hence, this dynamic platform requires a series of closed-loop designs for information collection and parameter adjustment to enable optimization and refinement. This section explores dynamic monitoring approaches for multidimensional signal data. Then, we elaborate on the applications of AI-assisted and sensor-based technologies in data processing, model prediction, and parameter design. These pathways will support and promote the achievement of adaptive feedback regulation within the system, with the aim of providing real-time

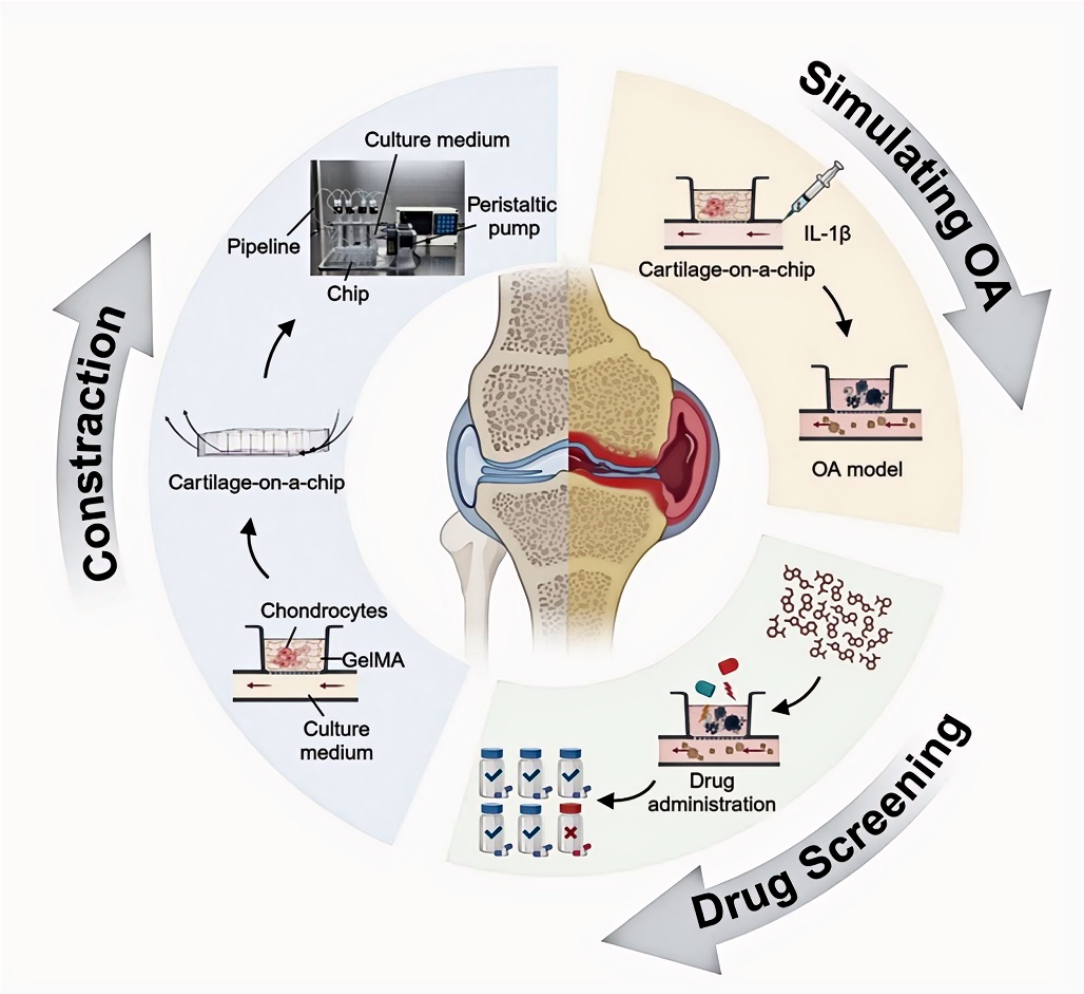


Figure 5. Schematic overview of cartilage organoids-on-a-chip for OA drug screening. This platform involves organoid construction, disease modeling, and drug screening. It enables the recapitulation of tissue microenvironment, disease pathogenesis, and drug response within an integrated microfluidic system.[83]

monitoring and AI assistance for organoid research platforms.

7.1. Signal monitoring and processing techniques

The core requirements of signal monitoring are real-time synchronization and accurate data acquisition. This section will elaborate on existing cutting-edge technical approaches for signal monitoring across three dimensions: mechanical, metabolic, and immune.

Mechanical signal monitoring requires precise quantification of loading stimuli and visualization of cellular responses, such as compressive and shear loading strains and cytoskeletal tension. These signals can be monitored through flexible electronic sensors and live-cell mechanical imaging techniques. For example, Lyu *et al.*[86] designed a flexible electronic sensor with advantages including ultra-high sensitivity, topological matching, and wireless operation. This sensor has great suitability for precise monitoring of bone and cartilage organoids. In addition, Jiang *et al.*[87] designed a multidirectional strain sensor that made breakthroughs in high elasticity and anisotropy through segmented embedding of materials. The combination of these two is expected to achieve a balance between flexibility and mechanical strength of the sensors, thus integrating their respective advantages. The synergistic use of these two technologies could contribute to precise monitoring of complex loading stimuli. On the other hand, FRET-based molecular tension sensors can function in multiple aspects involving fabricating, imaging and, analyzing mechanical stimuli. Thus, this approach enables the detection of how cells sense and respond to the mechanical properties of their surrounding microenvironment.[88]

Metabolic signals serve as critical hubs for dynamic functional coupling. Given the diverse metabolite components in the microenvironment that cells depend on,

it is necessary to monitor subtle fluctuations on timescales ranging from minutes to hours. Electrochemical biosensor integrated arrays can offer considerable advantages in achieving continuous, high-resolution, quantitative, and *in situ* acquisition of energy metabolites such as oxygen, lactate, and glucose. By controlling culture conditions, this technique has the potential to standardize the cellular microenvironment at the macroscopic level.[89] Furthermore, cutting-edge multi-omics strategies collectively provide information on biomolecular interactions within the tissue microenvironment, such as high-resolution mass spectrometry imaging and mass spectrometry–based proteomics and lipidomics. Additionally, these technologies jointly map the spatial distribution and gradient heterogeneity of metabolites from a holistic perspective.[90] Notably, the combination of high-resolution rapid information capture and holistic information gradient construction provides a foundation for further model prediction. Moreover, it is expected to facilitate the integration of machine learning and AI approaches for technological iteration.

The key to monitoring the immune module lies in tracking the migration, activation, and contact behaviors of immune cells. Likewise, their cytokine output during dynamic interactions with osteocytes and chondrocytes is significant. CELLECT is a contrastive embedding learning method suitable for large-scale and efficient cell tracking, capable of monitoring how cells dynamically migrate, interact, and function. By tracking processes such as inflammatory responses and tissue-specific immunity, it contributes to quantitative analysis of cell behavior in pathological and immunological contexts.[91] Kok *et al.*[92] developed a semi-automated cell tracker based on machine learning with convolutional neural networks, providing a new pathway to address the bottlenecks of high cell density and rapid cell movement in organoids. In addition, spatial transcriptomics can be applied to key joint compartments in osteoarthritis (such as cartilage, synovium, subchondral bone, and periarticular tissues). It assists in tracking regional gene expression patterns, pathogenic cell states, immune cell interactions, and signaling gradients that drive disease progression.[93] Combining machine learning pathways with multi-omics technologies is also expected to enable simultaneous detection of immune responses at multiple levels, includinhg cell behavior, gene expression, and signaling factors. Therefore, this multidimensional profiling of immune signals could provide a new tool for deciphering the complex interactions underlying immune responses.

Furthermore, considering the dynamic microenvironment of mechanical dysregulation, metabolic disturbance, and immune disruption in OA pathology, the monitored data are often fragmented and multimodal. Hence, it necessitates efficient fusion processing, including mechanical loading stimuli, spatial metabolic maps, and cell behavior trajectories. The core of data integration processing lies in the compatibility of different data types, such as time series, imaging, and omics. Further, the extraction of latent factors or network features with clear biological significance from the data can help with dimensionality reduction and interpretation.[94] Multi-omics factor analysis is a technique primarily used to compute the principal sources of variation in multi-omics datasets, capable of resolving axes of heterogeneity. Its processing capacity ranges from multiple modalities to a specific single data modality. Meanwhile, it also supports multiple downstream analyses, providing a core algorithmic framework for extracting shared variation factors.[95] Moreover, graph machine learning methods such as graph neural networks have distinct advantages in integrating multi-omics data and modeling complex biological relationships. These approaches represent an advanced paradigm for processing and interpreting multimodal organoid data.[96] The fusion and dimensionality reduction of multimodal data facilitate the interpretation of dynamically coupled signals, providing a usable information basis for intelligent closed-loop control. Thus, parameter adjustment and predictive modeling of organoid culture should be strongly supported by an understanding of the dynamic pathology of OA.

7.2. Artificial intelligence

In the process of organoid culture and simulation studies, large volumes of complex and heterogeneous datasets are typically generated. This feature poses considerable challenges to data analysis and processing capabilities. In recent years, AI has drawn increasing attention in multiple fields and has demonstrated substantial value and broad prospects in data processing, image analysis, and disease modeling. The principal techniques of AI include machine learning, deep learning, computer vision, and natural language processing. These technologies are capable of handling large-scale and high-throughput data types, especially suitable for the complex analysis of multi-omics data, including transcriptomics, genomics, epigenomics, proteomics, and metabolomics.[97] Furthermore, AI possesses excellent capabilities for evaluating and processing organoid models, such as standardized culture, trajectory simulation, and process automation. Obviously, AI will significantly improve the repeatability and precision of organoid models, meeting the precision requirements of regenerative medicine.[98]

A notable advantage of AI lies in its high-throughput image analysis capability, particularly the convolutional neural network (CNN) technology of deep learning. CNN is widely used for image recognition, image segmentation, and object detection. Deep residual learning is a common

approach within CNN, supporting dynamic monitoring and tracking of organoid culture. Extensive research demonstrates the notable efficiency and accuracy of CNN.[99,100] A representative example is the CNN-based OrganID designed by Matthews *et al.*,[101] which can identify, label, and track individual organoids under bright-field and phase-contrast microscopy. Remarkably, the automated quantification achieved 95% counting accuracy and 97% size-measurement accuracy.

Notably, the emergence of AI has substantially optimized parameter control in organoid-on-chip platforms. By adjusting parameters such as liquid flow rate, mechanical loading, and drug concentration, AI can adapt and regulate the microfluidic substance perfusion and mechanical cues, thus improving conditional controllability and microenvironmental simulation fidelity.[97] This strength makes it particularly suitable for simulating vascularization function and mechanical gradient induction. Through this technique, AI facilitates the integrated formation and functional coupling of osteochondral units and holds considerable potential in the field of osteochondral organoid-on-chip. In addition, AI-enhanced multi-organoid connected chip systems warrant further attention because they can be used for systemic metabolic kinetics studies. In future, these platforms hold immense potential in multi-organ combined expansion research and drug screening.[102]

In existing organoid research, AI technologies are used in the construction and design of multiple processes and have proved strong supporting capabilities. Key aspects that can be assisted by AI include material design[103] (selection of cells, hydrogels, and factors), model fabrication,[104] multimodal data acquisition (mechanical parameters, metabolomics, and cell lineage), data processing and evaluation,[105] system support,[106] and disease modeling and prediction.[107] Together, these aspects cover a complete chain of organoid construction—from design input and process monitoring to outcome evaluation and future prediction, which are expected to further evolve into a closed-loop system. As a tool for data analysis and model prediction with powerful capabilities and immense potential, AI has undoubtedly provided a major driving force for the transformative advancement of organoid technology. The further integration of AI and organoids holds broad opportunities for progress in dynamic and comprehensive analysis, as well as functional coupling and simulation of osteochondral organoids.

8. Conclusion

The osteochondral unit possesses a complex gradient structure, with numerous cell types and multiple coupled functions, exhibiting dynamic microenvironmental characteristics during growth, development, and OA pathology. These features pose enormous challenges to the integrated biomimetic construction of osteochondral organoids. Faced with such complex requirements, the demands for spatial cell arrangement, nutrient delivery, and growth factor regulation further increase the difficulty of organoid culture. Meanwhile, issues such as batch reproducibility and scalability remain. At present, bone or cartilage organoids continue to face challenges in recapitulating mechanical loading capacity, complex vascularization, ECM, cellular composition, and model size. Besides, as the next goal, the integrated construction of osteochondral organoids remains difficult to achieve at present. The core mainly lies in their biomimetic capacity for multiple gradients and functional interactions. However, it is encouraging that the close integration and synergistic development of engineering technologies and organoids have created a turning point for addressing the above difficulties. The emergence of fabrication technologies such as 3D bioprinting and microfluidic chips has provided solutions to challenges in organoid construction, supporting pathways to structural gradient construction, biochemical factor delivery, and high-throughput production. Through a series of practical applications, the advantages and feasibility of these technologies have been increasingly demonstrated. In addition, the rapid development of AI has also generated a strong impetus for the transformation of organoid technology, with AI-assisted approaches progressively enabling more intelligent, precise, and personalized organoid cultivation processes and expanding their potential applications.

Given the complexity of integrated construction of osteochondral organoids, traditional cultivation approaches can hardly resolve the above bottlenecks. Cutting-edge engineering and integration technologies provide feasibility for modular design, which is expected to become the mainstream construction strategy for osteochondral organoids in future. This review focuses on three core gradients in the osteochondral microenvironment—mechanical, metabolic, and immune. Then, in conjunction with cutting-edge research, we elaborate on the programming strategies and feasibility of related signals, aiming to provide insights for the induction and formation of complex microenvironments and multiple gradients in organoid models. The goal of this design is intended to achieve functional regeneration of this unit. On this basis, existing integration technologies are introduced, including 3D bioprinting, organ-on-chip, and organoid assembloids. These techniques are expected to functionally couple individual modules and thereby further enhance the feasibility and scalability of modular organoid construction. Next, this paper introduces advanced signal detection, auxiliary analysis, and AI technologies. With considerable potential, such approaches are expected to

facilitate precision and personalization, further improving the scale-up of organoid culture. Based on the above considerations, this review summarizes a promising modular design for osteochondral organoids and proposes a corresponding framework (Table 3), with the aim of advancing the development of osteochondral organoids.

Table 3 illustrates a potential framework of the modular design for osteochondral organoid construction. This table summarizes the proposed modular framework as a stepwise workflow, proceeding from spatial zoning to the mechanical, metabolic, and immune modules, and ending with coupled validation. For each step, it specifies the biological significance and the key parameters to be controlled, thereby translating the conceptual framework into concrete design decisions. The five steps are organized in a logical sequence, with the final coupling-validation step serving as the checkpoint to assess the compatibility and integration of the three signaling modules. Together, these principles and parameters provide a practical reference for the rational construction of osteochondral organoids.

It must be acknowledged that modular design based on engineering integration technologies has shown certain feasibility, but owing to current technical bottlenecks, a considerable gap remains between their theoretical foundation and practical implementation. The construction of mechanical, metabolic, and immune gradients, as well as more complex multidimensional coupling systems, remains challenging. In addition to the existing bottlenecks in bone and cartilage organoid technologies, their integration remains constrained by several limitations that cannot be overlooked, including insufficient immune functionality in gradient 3D bioprinting, size limitations of assembloids, and persistent challenges in achieving effective vascularization. Moreover, the cross-integration of multiple technologies may entail underlying conflicts in conditions. On the other hand, issues regarding standardization and reproducibility remain. Furthermore, a translational gap still exists between *in vitro* model construction and *in vivo* implantation and repair, limiting the clinical maturity and practical application of these technologies. In summary, although the integrated construction of osteochondral organoids offers advantages in terms of structural and functional integration, it also presents numerous and formidable challenges, and the transition from feasibility to technological maturity remains a long-term endeavor. Nevertheless, with the standardization and interdisciplinary integration of technologies, as well as advances in AI technology, osteochondral organoid technology is expected to continue evolving toward greater maturity and broader application in clinical research, thereby providing support for the treatment of OA and the advancement of regenerative medicine.

Table 3. Several principles and key parameters of modular design

Step	Significance	Key parameters
1. Spatial zoning	Provide spatial carrier for signals, cues, and gradients	Layer thickness; Porosity; Tidemark location; Interface width
2. Mechanical module	Key determinant of cell fate; Stiffness support for structure	Matrix stiffness; Loading range; Stress-relaxation time; Loss modulus
3. Metabolic module	Establish the oxygen gradient; Nutrient delivery and waste transport	Transitional oxygen pressure; Glucose/lactate levels; Dynamic/static nutrient supply mode
4. Immune module	Immune competence; Disease-modeling simulation; Potential personalized application	Immune cell type and ratio; Identity and dose of inflammatory cytokines; Immune-privilege cues
5. Coupling validation	Close the design loop and confirm the outputs	Tri-readout; Inter-batch reproducibility

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Conflict of interest

The authors declare they have no conflict of interest.

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Supervision: Yixing Ren, Xiaoming Li
Writing–original draft: Zihan Xu
Writing–review & editing: Zihan Xu, Xiaoming Li

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

Further disclosure

Generative AI and AI-assisted technologies were only be used in initial draft translation and language polishing to improve readability and language.

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