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REVIEW ARTICLE

Advances and challenges in three-dimensional bioprinting of bone organoids: Materials, techniques, and functionalization strategies

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Abstract

The use of three-dimensional (3D) bioprinting to construct bone organoids holds significant promise in bone tissue engineering due to its potential to replicate complex structures for research and regenerative medicine. This technology enables the creation of precise 3D structures through layer-by-layer deposition of bioinks guided by digital models. However, challenges remain in achieving functional bone organoids, especially in bioink design, vascularization, and cell viability preservation. To address these issues, various printing techniques such as extrusion, inkjet, light-curing, and microfluidic printing have been explored, but further advances are needed to improve the quality and functionality of printed bone organoids. This review assesses the current state of research on the application of 3D bioprinting techniques for the construction of bone organoids, focusing on the selection of bioinks, scaffold materials, and the role of cells and growth factors. Despite notable progress, significant challenges remain in optimizing the mechanical properties of bioinks, enhancing vascularization, and mimicking the dynamic physiological environment of bone tissue. The main objective of this study is to explore the technical challenges and opportunities in the construction of functional bone organoids through 3D bioprinting, aiming to provide insights into future directions for overcoming these obstacles and improving bone tissue regeneration applications.

Keywords: Bone organoids; Bioink; Three-dimensional bioprinting; Tissue engineering; Vascularization

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1. Introduction

Bone tissue, as the body's structural support system, serves multiple functions such as protecting internal organs, maintaining body shape, facilitating movement, and regulating mineral metabolism. However, the skeletal system often faces challenges from injury, aging, and disease, resulting in bone defects and loss of function. Bone defects not only seriously affect patients' quality of life but also place a heavy burden on healthcare systems and society. With the global aging population, the incidence of skeletal diseases is increasing, especially large-scale bone defects due to fractures, tumors, or infections, which have become a critical concern in current clinical medicine.[1]

Bone organoid is a three-dimensional (3D) *in vitro* model that has recently emerged from advances in organoid technology and tissue engineering, aiming to mimic the structure, function, and microenvironment of natural bone tissues and to provide a more accurate platform for investigating bone-metabolism-related disease mechanisms, drug screening, and regenerative medicine applications.[2]

Three-dimensional bioprinting is a technology that fabricates 3D structures through the layer-by-layer deposition of biomaterials guided by digital models. This approach not only enables the printing of biological scaffolds but also simultaneously loads cells, drugs, and other biomolecules into the scaffolds, thereby broadening its applications in tissue engineering and regenerative medicine.[3] In bone tissue engineering, the application of 3D bioprinting technology has been proven to provide personalized and precise solutions for bone defect repair. Printing bone scaffolds with tailored shapes, pore structures, and mechanical properties enhances their ability to support bone cell growth and differentiation, thereby promoting new bone formation. In addition, 3D bioprinting technology can be combined with stem cell technology, genetic engineering technology, and advanced biomaterials to achieve complete bone tissue regeneration.[4]

Currently, despite the remarkable progress of 3D bioprinting technology in bone tissue engineering, there are still multifaceted challenges in constructing bone organoids. These include designing bioinks with optimal biocompatibility and appropriate mechanical properties, engineering large-scale vascular networks in organoids, maintaining cell viability throughout the printing process, and ensuring the long-term stability and biological functionality of the printed organoids.[5,6]

This article focuses on the application and advancements of 3D bioprinting technology in the construction of bone organoids. It provides a comprehensive review of the development of this technology within the field and analyzes the current challenges and future directions. The article firstly introduces the basic principles of 3D bioprinting, the types of technologies and their applications in bone organoid printing, followed by a discussion on the design requirements of bone organoids, the selection and optimization of cell carrier ink, the challenges faced by vascularization, and the optimization of the structure during the printing process. Lastly, the article analyzes the limitations of existing technologies and explores prospective developments, such as novel biomaterials, multifunctional bioinks, and the clinical translation of this technology. The aim of this review is to present the latest advances in 3D bioprinting applications for bone organoids, offering valuable insights for researchers and clinicians, and to serve as a useful reference to guide future research and clinical practice.

2. Overview of three-dimensional bioprinting technologies

2.1. Principles of three-dimensional bioprinting

Unlike traditional 3D printing, the core of 3D bioprinting extends beyond precise structural fabrication to emphasize cell viability, tissue growth, differentiation, and ultimate biological function.[7] The fundamental steps include digital design, ink selection, layer-by-layer printing, and post-printing culture (Table 1). The process begins with digital model design, which translates medical data into 3D structures through the use of computer-aided design software.[8] In computer-aided design software, designers are able to customize the scaffolds of bone organoids according to specific requirements. For example, scaffold porosity must balance mechanical support with adequate space for cell growth.[9] Bioink, the core component of 3D bioprinting, primarily consists of cells, support materials, and growth factors. Choosing the right bioink is crucial for printing high-quality bone organoid scaffolds (Figure 1). Suitable bioinks must meet several requirements, including biocompatibility, mechanical properties, degradability, and the ability to support cell growth and differentiation.[10,11] Following bioink selection, the 3D model is sliced into

Table 1. Key stages and parameters in bone organoid construction

Stage	Main operations	Key parameters	Influencing factors	Reference
Digital model design	Computer-aided design modeling, pore optimization	Porosity, mechanical strength	Data resolution, software parameters	8
Ink selection	Mixing cells, biomaterials, and growth factors	Viscosity, rheology, and biocompatibility	Cell type, biomaterial properties	10
Three-dimensional printing	Layer-by-layer deposition, crosslinking, and curing	Printing resolution, curing speed	Printing speed, environmental temperature	12,13
Post-culturing	Cell culture, dynamic stimulation	Cell proliferation, differentiation, and vascularization	Mechanical stimulation, growth factors	15,16,18

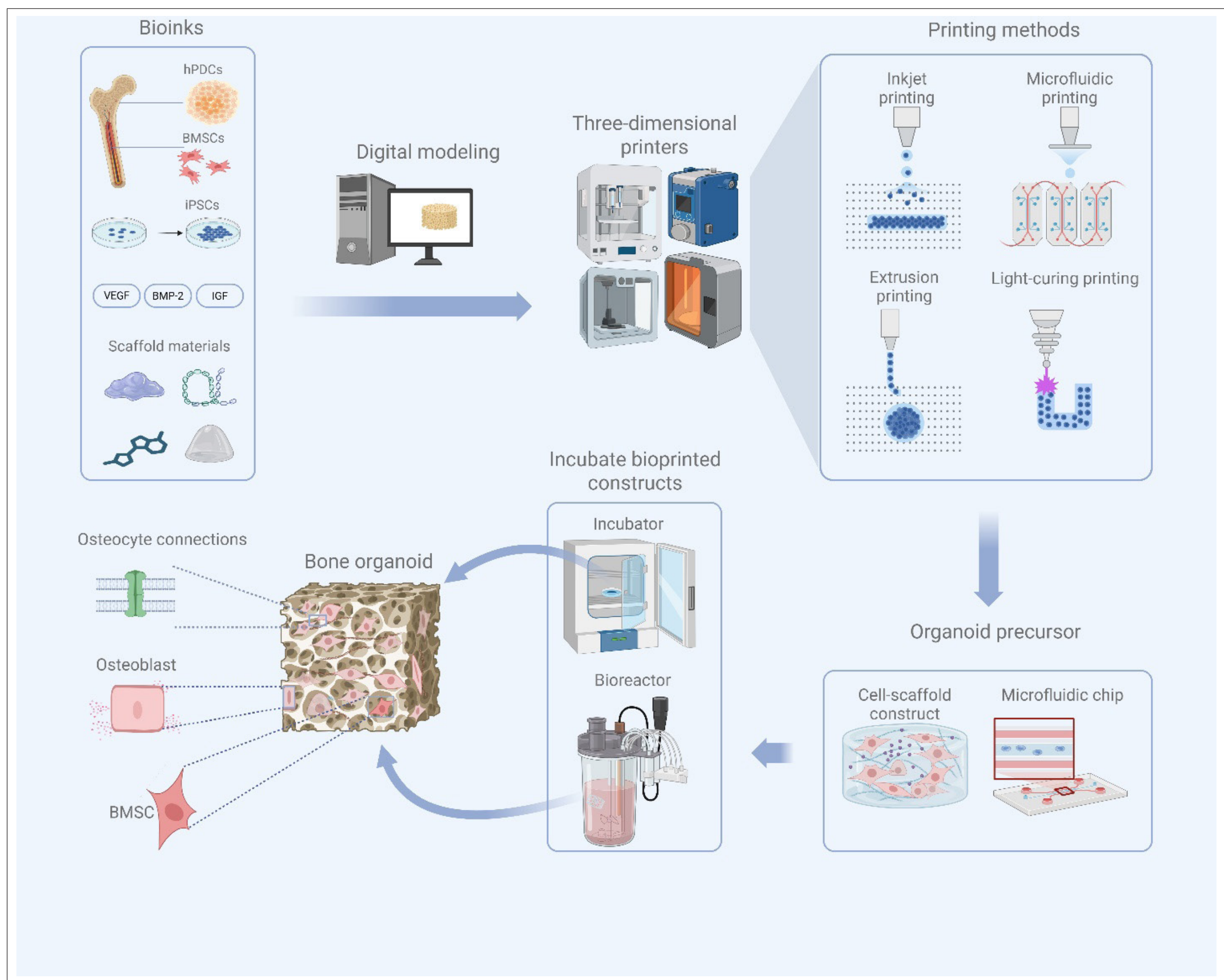

Figure 1. Workflow of three-dimensional (3D) bioprinting for bone organoid construction. The process begins with the selection of bioinks, which include cells, growth factors, and biomaterials. Subsequently, a suitable printing method is chosen based on computer-assisted design modeling. The printed bone organoid precursors are then placed into bioreactors or incubators for further maturation. Finally, the process results in the cultivation of fully matured bone organoids. Schematic diagram created by the authors. Abbreviations: BMSCs, bone marrow mesenchymal stem cells; BMP-2, bone morphogenetic protein-2; hPDCs, human periosteum-derived cells; IGF, insulin-like growth factor; iPSCs, induced pluripotent stem cells; VEGF, vascular endothelial growth factor.

a series of two-dimensional slices, which are printed sequentially. Each layer is printed to ensure structural stability and cellular viability. After each layer is printed, the new ink layer forms a bond with the preceding layer, and the quality of the bond between layers directly affects the overall stability and structural integrity of the print.[12] Certain 3D bioprinting technologies perform a curing process after each layer to stabilize the morphology and mechanical properties of the print. The curing process transforms the bioink from a liquid to a solid state through ultraviolet (UV) light, laser, calcium ions cross-linking, or chemical agents to ensure good bonding between each layer. Poor bonding between layers may lead to fragile scaffolds and affect the normal growth and distribution of cells, especially in biological scaffolds.[13] Therefore, the temperature, humidity, printing speed, and curing process during the printing process must be precisely controlled to ensure proper bonding between each layer. Scaffolds after 3D bioprinting are usually placed in cell culture tanks or bioreactors that mimic the *in vivo* environment by supplying the cells with the required nutrients, oxygen, and growth factors for organoid development.[14,15] In addition, dynamic culture systems such as rotating bioreactors, fluidic culture systems, and microfluidics can be used to provide cells with the required fluid shear, pressure, and flow environment[16] (Figure 1). In 3D bioprinting, scaffolds

serve not only as physical supports for cell growth but also as dynamic structures whose degradation synchronizes with the maturation of bone organoids. As new tissue grows and matures, the scaffold material gradually degrades and is replaced by native tissue. This degradation process must be matched with tissue regeneration to ensure continuous support until the scaffold is completely replaced.[17,18] Culturing bone organoids involves complex, long-term processes such as cell proliferation, differentiation, mineralization, and vascularization. Therefore, it is crucial to select scaffold materials that can remain stable over time and not be rapidly degraded to ensure a successful formation of bone organoids.

2.2. Key three-dimensional bioprinting technologies

Extrusion printing is a widely adopted 3D bioprinting technology that uses methods such as pneumatic pressure, mechanical extrusion, or screw drive to propel the bioink to be extruded from a nozzle and deposited onto the print substrate.[19] Once printed, the bioink typically needs to be cured either physically (e.g., by cooling) or by chemical cross-linking to form a stable structure. This printing technique is capable of handling a wide range of materials, including natural hydrogels and synthetic polymers.[20,21] By adjusting the formulation of the bioink, the mechanical properties, degradation rate, and porosity of the scaffold can be customized to meet the needs of different bone organoids printing.[22] However, conventional extrusion printing requires high-temperature melting of biomaterials, which can cause severe thermal damage to the cells in the bioink. Essential cellular components, such as proteins, lipid bilayers, and nucleic acids, are susceptible to denaturation at high temperatures, leading to cell death or loss of function. Even when cells undergo pretreatment prior to printing, thermal stress may still cause irreversible damage. To minimize the effects of high-temperature materials on cells, researchers have increasingly employed low-melting-point biomaterials for printing, thereby reducing thermal damage to cells.[23] Among various 3D bioprinting techniques, extrusion printing technology is able to maintain a high cell density during the printing process. When constructing bone organoids, this high cell density helps to promote cell-to-cell interactions, which are essential for cell differentiation and functionalization. Although high-viscosity bioinks can support high cell density, they may cause mechanical damage to cells during extrusion.[24] Therefore, optimizing the balance between bioink viscosity, fluidity, and cell viability, while ensuring higher cell density, remains a significant challenge in extrusion printing.

Inkjet printing technology utilizes physical principles, such as thermal or piezoelectric jets, to precisely eject liquid bioink through nozzles, forming minute droplets that are deposited onto a print substrate following a preset path. The size and frequency of the droplets are controlled by the printer.[25] Through layer-by-layer deposition, these droplets accumulate to form a 3D structure. Following each layer's deposition, cooling, cross-linking, or other curing methods are often required to ensure the stability of the printed structure. This technology is capable of producing micron-sized droplets with precise control over cell and material deposition, making it suitable for fabricating tiny pores and fine structures. Consequently, inkjet printing is ideally suited for the printing of high-throughput cell arrays that can be used for drug screening, toxicity testing, and drug response studies. In addition, it enables precise deposition of endothelial cells to progressively construct vascular endothelial layers and microtubular structures, resulting in vascularized networks.[26] Vascularization is a key component in the construction of bone organoids, as tissues lacking vascular support cannot receive sufficient oxygen and nutrients. By printing microvascular networks, inkjet printing technology may supply blood perfusion to printed bone and cartilage scaffolds, thereby accelerating tissue growth. To ensure smooth droplet ejection and deposition, bioinks with low cell density are typically required.[27,28] In bone organoid construction, low cell density scaffolds may lead to slower cell proliferation and differentiation, complicating organoid development. Meanwhile, droplet deposition can cause local aggregation, potentially resulting in scaffolds with poor pore connectivity or non-uniform pore sizes, especially when printing scaffolds with larger areas. Addressing these challenges requires optimization of printing parameters and bioink formulations to improve the quality and functionality of the printed scaffolds.

Light-cured bioprinting employs UV or visible light to irradiate photosensitive resins or light-curing hydrogels, triggering photocrosslinking reactions that cure the material into mechanically strong structures. This technology has exceptional printing accuracy, often achieving micron-level resolution. The ability to print at high resolution ensures precise detail in bone organoids scaffolds, promoting cell growth and functional recovery of tissues.[29] Beyond single-cell-type printing, light-cured printing can co-print multiple cells by adjusting printing parameters and material formulations.[30] Further development of this technology is expected to promote vascularization of bone organoids by simultaneously printing different cell types, such as osteoblasts and vascular endothelial cells, in the same scaffold. The intensity of the light source and the exposure time directly affect the curing speed and depth of the photosensitive resin, which in turn determines the precision of light-cured printing.[31] Higher intensity can accelerate the curing

process, but may also lead to uneven curing of the surface, while lower intensity slows curing, affecting the accuracy of the print. The exposure time for each layer needs to be precisely adjusted according to the material properties and layer thickness. Excessive exposure causes over-curing, impairing the adhesion of subsequent layers; conversely, insufficient exposure results in under-curing, affecting the bonding between layers. At the same time, excessive light intensity or prolonged exposure may damage the cells in the bioink, leading to cell death or functional impairment.[32] Therefore, it is critical to adjust the intensity and exposure time of the light source to ensure uniform curing of each layer while preserving cell viability and function, which are critical for the precise construction of complex structures such as bone organoids.

Depending on the light-curing printing technology, the type of light source and curing process vary slightly. Common light-cured printing technologies include stereolithography (SLA), digital light processing (DLP), and confocal lithography. SLA technology uses a laser beam as the light source, which scans the surface of a liquid photosensitive resin to locally initiate curing. With each scan, the laser illuminates the surface of the resin, completing the curing of a single spot.[33] By precisely controlling the laser's scanning path and exposure time, the point-by-point curing forms the structure of each layer, enabling SLA to achieve very high printing accuracy. DLP technology uses a digital light source combined with a liquid crystal display to irradiate and cure the entire layer at once by projection. Unlike SLA's laser point-by-point curing, DLP uses projection technology to cure the entire layer more quickly, making it suitable for printing large-area structures. However, it may exhibit slightly lower accuracy than SLA.[32,34] Confocal lithography combines light-curing printing and confocal microscopy to enable layer-by-layer curing at high precision. Using the light source of a confocal microscope, each part of the photosensitive material is precisely illuminated, ensuring precision and stability of the print. This technology provides greater precision and more intricate detail and is particularly suited for printing microstructures and microvascular networks. Despite the advantages of DLP's full-layer curing, light-cured printing methods are still challenged by slower printing speeds compared to other 3D printing technologies.

Microfluidic printing combines microfluidic technology and bioprinting by precisely controlling fluid flow and droplet generation in microchannels. Utilizing fluid dynamics principles such as pressure difference, surface tension, and capillary action, microfluid printing enables precise layer-by-layer deposition of bioink at the target location to construct complex 3D structures layer by layer.[35–37] The microfluidic channels are designed to precisely control the speed, direction, and flow rate of the liquid flow to ensure stable deposition of bioink during printing, while droplet generation units convert bioink into tiny and uniform droplets by adjusting flow parameters. These droplets are precisely deposited onto the target area during the printing process, allowing for the precise positioning of cells and support materials.[38] The microfluidic channel is able to effectively regulate the frequency and position of droplet generation to ensure uniform distribution of cells in the support. The size, shape, and arrangement of the microfluidic channels directly affect the uniformity of cell deposition and the biological properties of the final printed structure. By optimizing the channel design, microfluidic printing may enable the co-printing of different cell types, ensuring that multiple cells are distributed in the scaffold in a predetermined pattern that promotes cell-cell interactions and functionalization. This precise cell alignment and material deposition can provide ideal support for the construction of complex tissues such as bone organoids. In addition, microfluidic printing technology is able to mimic complex physiological microenvironments in the body, such as blood flow, oxygen, and nutrient supply, through the tailored design of microchannel systems.[39,40] These microenvironmental factors are critical for cell growth, differentiation, and functional recovery. In bone organoid construction, microfluidic printing not only provides ideal physiological conditions but also simulates blood flow and nutrient exchange by regulating fluid flow, ensuring adequate oxygen and nutrient delivery within the scaffold to promote bone tissue growth and maturation. Additionally, microfluidic printing technology enables the controlled release of growth factors by precisely controlling the fluid flow in microchannels. These growth factors can promote the proliferation, differentiation, and mineralization of bone cells. The microfluidic system is able to precisely regulate the release rate and spatial distribution of growth factors according to the scaffold design, thus further accelerating the construction and functional recovery of bone tissue. However, this technology relies on complex microfluidic chips and precise fluid control systems, resulting in high equipment costs and high operational requirements.[41] In addition, optimizing the microchannel design to ensure uniform cell distribution and stable growth factor release still requires further research. These limitations have hindered the widespread application of microfluidic printing in large-scale bone tissue engineering (Table 2).

2.3. Selection and requirements for bioinks

Bioinks are materials specifically designed to be used in bioprinting and usually consist of a matrix material, cells, and growth factors.[42] The scaffolding material provides the supporting structure and space, the cells serve as the

Table 2. Comparison of three-dimensional bioprinting technologies

Technology	Principle	Advantages	Disadvantages	Applications	Outlook	Reference
Extrusion printing	Pushes bioink through a nozzle	High cell density, suitable for many materials	High temperatures may damage cells	Bone scaffolds, high cell density tissues	Low-melting materials to reduce cell damage	19
Inkjet printing	Sprays ink in droplets layer by layer	High precision, good for small structures	Low cell density, poor pore connectivity	Cell arrays, vascular networks	Improve pore structure and cell proliferation	25,26
Light-cured printing	Uses light to solidify photosensitive materials	High precision, supports multi-cell printing	Light exposure can damage cells	High-precision structures; complex organoids	Better light control to maintain cell viability	29,31
Microfluidic printing	Controls fluid in microchannels for precise deposition	Precise control of cell placement and growth	Complex design, requires precise fluid control	Three-dimensional structures; simulate physiological environments	Optimize channels for growth factor release	35–37

basic units for building tissues, and the growth factors promote the growth, differentiation, and mineralization of bone tissue by regulating cellular behavior. Depending on the printing technology, the formulation and structure of bioinks may vary.

2.3.1. Scaffold materials

Scaffolding materials constitute a core component of cell carrier bioinks, playing a critical role in providing structural support and a 3D growth environment for cells. They mimic the extracellular matrix (ECM) of natural tissues to create a suitable microenvironment for cells, promoting cell growth, proliferation, differentiation, and functional expression.[43] The selection of scaffold material directly affects the mechanical properties of bone organoids, their degradation rate, bone tissue regeneration efficiency, and the stability and functionality of the final printed structure. Common scaffold materials can be categorized into hydrogels, synthetic polymers, inorganic materials, and composites, each offering unique advantages and disadvantages.

Hydrogels are among the most commonly used scaffolding materials in 3D bioprinting, providing an environment that closely mimics ECM due to their high water content and excellent biocompatibility. Hydrogels can be categorized into two types: natural hydrogels and synthetic polymers. Natural hydrogels, such as gelatin, sodium alginate, and collagen, have good biocompatibility and degradability and offer an ideal environment that supports cell growth and proliferation.[44,45] Gelatin, a natural macromolecule derived from collagen hydrolysis, promotes cell adhesion and proliferation, owing to its biocompatibility and biodegradability; however, its mechanical properties are insufficient, and its rapid degradation may render it unsuitable for long-term support in bone organoids. Sodium alginate, a natural polysaccharide, can be combined with cross-linking agents such as calcium salts to regulate its mechanical properties, making it suitable for bone organoid printing.[46,47] Collagen, as the primary matrix protein in bone tissue, holds potential to promote bone regeneration, although its mechanical properties are weak and insufficient to mimic the hardness of natural bone. Synthetic polymers, with tunable mechanical properties and degradation characteristics, are commonly used to enhance the structural strength of scaffolds.[48] However, they are usually less biocompatible than natural materials. Polyvinyl alcohol has good biocompatibility and mechanical properties, but has a slow degradation rate and poor cellular support. Polyvinylpyrrolidone has moderate mechanical properties and biocompatibility, but has poor degradation properties, and usually needs to be compounded with other degradable materials. By integrating natural hydrogels with synthetic polymers, composite hydrogels are able to combine the advantages of both material classes, retaining the excellent biocompatibility and cellular support of the natural materials while imparting the controlled mechanical properties and degradation characteristics of the synthetic materials.[45,49] The main advantage of these composites lies in the ability to adjust their composition and properties, enabling optimization of biocompatibility, mechanical properties, and degradation rates in bone organoids printing. By adjusting the ratio of natural to synthetic materials, the desired performance can be achieved in different applications. The tunability of composite hydrogels gives them a unique advantage, facilitating the printing of a wide range of bone organoids with optimized biological and mechanical characteristics, thereby promoting their further development in clinical applications. Inorganic materials such as hydroxyapatite (HAP) and tricalcium phosphate are widely used in 3D bioprinting, enhancing the mechanical strength of the scaffolds and promoting the

mineralization of bone tissue.[50,51] HAP, being a key element of the natural bone matrix, promotes the attachment, proliferation, and differentiation of osteoblasts and enhances the bioactivity of the bone organoids; whereas tricalcium phosphate promotes bone mineralization and provides structural support that closely resembles natural bone, fulfilling the requirements for bone regeneration (Table 3).

2.3.2. Cells and growth factors

Human periosteum-derived cells (hPDCs) are a population of cells isolated from the periosteum and possess specific biological properties. These cells are obtained through the enzymatic digestion of periosteal tissues and can be cultured and expanded for research and applications. hPDCs possess multipotent differentiation potential, and under suitable culture conditions, they can differentiate into osteoblasts and chondrocytes, contributing to bone and cartilage formation and repair.[52] In bone tissue engineering, hPDCs are an ideal cell source due to their ability to construct bone organoids or repair bone defects. These cells can also form spheroids or organoids of varying sizes. For instance, when cultured in customized agarose microtiter wells, their diameter can be controlled, ranging from 100 to 150 μm to approximately 300 μm.[53] This size modulation is crucial for 3D bioprinting, as different application scenarios may require different sizes of tissue constructs. During spheroid or organoid formation, hPDCs establish 3D tissue units characterized by intercellular junctions and ECM secretion.[54] These structurally complex spheroids or organoids can be used as basic building blocks for 3D printing, enabling their precise arrangement and assembly into bone organoids with enhanced structural complexity and biological function.

Bone marrow mesenchymal stem cells (BMSCs) are pluripotent stem cells derived from bone marrow, capable of differentiating into various cell types, particularly those of bone, cartilage, and adipose tissues.[55,56] BMSCs have a wide range of applications in tissue engineering, regenerative medicine, and cellular therapies due to their excellent potential for self-renewal, proliferation, and differentiation. BMSCs have strong proliferative capacity and can expand sustainably *in vitro*. Despite the possibility of cellular senescence and differentiation limitation during long-term culture, BMSCs can still maintain proliferative activity for a long time.[57] With appropriate culture conditions, BMSCs can be obtained in large numbers in long-term culture, making them ideal for use as cells for constructing bone organoids. In addition, BMSCs are highly adaptable, responding to different culture conditions and external stimuli during long-term culture. For example, in osteogenic and chondrogenic tissue formation, the culture environment can be adjusted to enhance their differentiation potential.[58] This characteristic allows BMSCs to exhibit considerable versatility in the construction of bone organoids.

Induced pluripotent stem cells (iPSCs) are adult cells that have been transformed into stem cells with multidirectional differentiation potential through genetic reprogramming. They are capable of differentiating into various cell types *in vitro*, including osteoblasts, chondrocytes, and osteoclasts, making them an ideal cellular source for bone organoid construction.[59] Given that iPSCs can be derived from the patient's own cells, they exhibit low immunogenicity and can be used for personalized therapy. In addition, iPSCs can be effectively differentiated into osteoblasts through optimized differentiation conditions, which can be used to construct functional bone tissues in combination with scaffolding materials for bone defect repair or bone organoid development.[60] However, challenges remain regarding the differentiation efficiency and stability of iPSCs, as genetic mutations introduced during reprogramming can compromise their long-term stability and safety.[61] Therefore, it is necessary to further optimize the differentiation induction technique of iPSCs and address issues such as genetic instability to promote their wide application in bone organoid construction.

Human umbilical vein endothelial cells (HUVECs) play a pivotal role in bone organoid vascularization due to

Table 3. Characteristics of scaffold materials for bone organoids

Type	Main components	Advantages	Applications	Reference
Natural hydrogels	Gelatin, alginate, hyaluronic acid	High biocompatibility, biodegradable	Cell scaffolds, bioinks	44–47
Synthetic polymers	Polyvinyl alcohol, polyvinylpyrrolidone	High mechanical strength, controllable degradation	Bone scaffolds, cartilage repair	49
Inorganic materials	Hydroxyapatite, calcium phosphate	Promote bone mineralization, good mechanical properties	Bone tissue repair	50,51
Composite materials	Natural hydrogels + ceramics or synthetic polymers	Combine bioactivity and mechanical properties	Composite bone tissue engineering	45

their strong angiogenic potential and compatibility with osteogenic systems. In both direct and indirect co-culture models, HUVECs retain endothelial lineage identity while promoting osteogenic differentiation in BMSCs through paracrine signaling.[62] This reciprocal interaction enhances vascularized tissue formation and is critical for the development of functional bone constructs. In 3D bioprinting strategies, HUVECs can be incorporated into bioinks and co-printed with BMSCs, facilitating their spatial organization along scaffold pores or printed microchannels. These cells can self-organize into tubular networks *in vitro*, recapitulating early vasculature development. By forming such prevascular structures, HUVECs not only improve nutrient transport but also help establish a regenerative microenvironment essential for bone matrix deposition.[63,64] The integration of HUVECs into bioprinted constructs has thus become a cornerstone technique in current vascularization approaches for bone organoids.

Growth factors play multifaceted roles in bone organoids. They not only stimulate the proliferation and differentiation of osteoblasts but also participate in several processes such as bone mineralization, angiogenesis, and tissue remodeling. Bone morphogenetic proteins (BMPs), especially BMP-2 and BMP-7, have been shown to significantly promote the osteogenic differentiation of BMSCs and are widely used in bone regeneration and repair.[65] In addition, osteoblast growth factors are also able to promote the proliferation of osteoblasts, which, in turn, improves the efficiency of bone organoid construction.[66] Mineralization is a key process in bone formation and bone organoids, regulated by growth factors that modulate ECM synthesis and mineral deposition. BMPs facilitate bone formation by stimulating ECM secretion from osteoblasts and promoting mineralization. Transforming growth factor-β and insulin-like growth factor-1 also have a beneficial effect on the synthesis of bone matrix by activating the relevant signaling pathways that enhance the ability of osteoblasts to synthesize collagen and minerals.[67,68] Thus, growth factors are essential for mineralization, especially during the maturation phase of bone organoids. Vascularization poses a significant challenge in constructing bone organoids, particularly for large-scale organoids. The formation of vascular networks is essential to ensure adequate oxygen and nutrient supply to the bone tissue. Vascular endothelial growth factor is a key factor in promoting angiogenesis. It promotes blood supply to bone tissues by facilitating the proliferation and migration of endothelial cells as well as the formation of new blood vessels.[69] The lack of an effective vascular network can result in bone organoids not receiving sufficient nutrients and oxygen, affecting their growth and function. In addition, the construction of bone organoids not only depends on cell proliferation and differentiation but also requires tissue remodeling. Matrix metalloproteinases play an important role in this process by degrading the ECM, providing space for cell migration and growth, and facilitating tissue repair processes.[70] In bone organoids, precise regulation of matrix metalloproteinases promotes new bone formation and accelerates the maturation of bone organoids. However, the clinical application of growth factors still faces certain challenges, such as possible side effects, stability issues, and delivery efficiency. Therefore, the development of improved delivery methods is necessary to optimize their effects in bone organoid engineering.

3. Design and construction of bone organoids

3.1. Basic principles of bone organoid bioprinting

3.1.1. Hierarchical structure

The natural structure of bone tissue is highly hierarchical, and its structural complexity and functionality enable bone tissue to provide strong mechanical support while meeting biological demands. When designing bone organoids, it is important to mimic the hierarchical nature of natural bone tissue as closely as possible to ensure mechanical properties, function, and bioactivity (Figure 2).

Cortical bone provides strength and rigidity to the skeleton and is usually located on the outer surface of bones, characterized by a dense structure. In contrast, cancellous bone provides a larger surface area and microchannels, enhances the elasticity of the bone tissue, supports vascularization, and promotes cell attachment and proliferation.[65,71] Typically, cortical bone regions require higher mechanical strength and rigidity and are, therefore, constructed using materials with higher modulus of elasticity and greater durability. The cancellous bone region, on the other hand, has greater porosity and smaller pore sizes, forming a "honeycomb"-like structure that provides space for cells to grow and supports the formation of a vascular network. The pore structure of the cancellous bone must be precisely designed to ensure proper cell growth, proliferation, differentiation, and vascularization.[72] Materials with greater porosity and better elasticity are often used to mimic the properties of cancellous bone. The use of 3D printing technology allows precise control of the pore structure, thereby improving cell viability and function within this region.[73]

Cartilage tissue functions primarily by reducing friction, absorbing shock, and supporting skeletal motion. When constructing cartilage organoids, the design should focus on the mechanical properties of cartilage, such as low

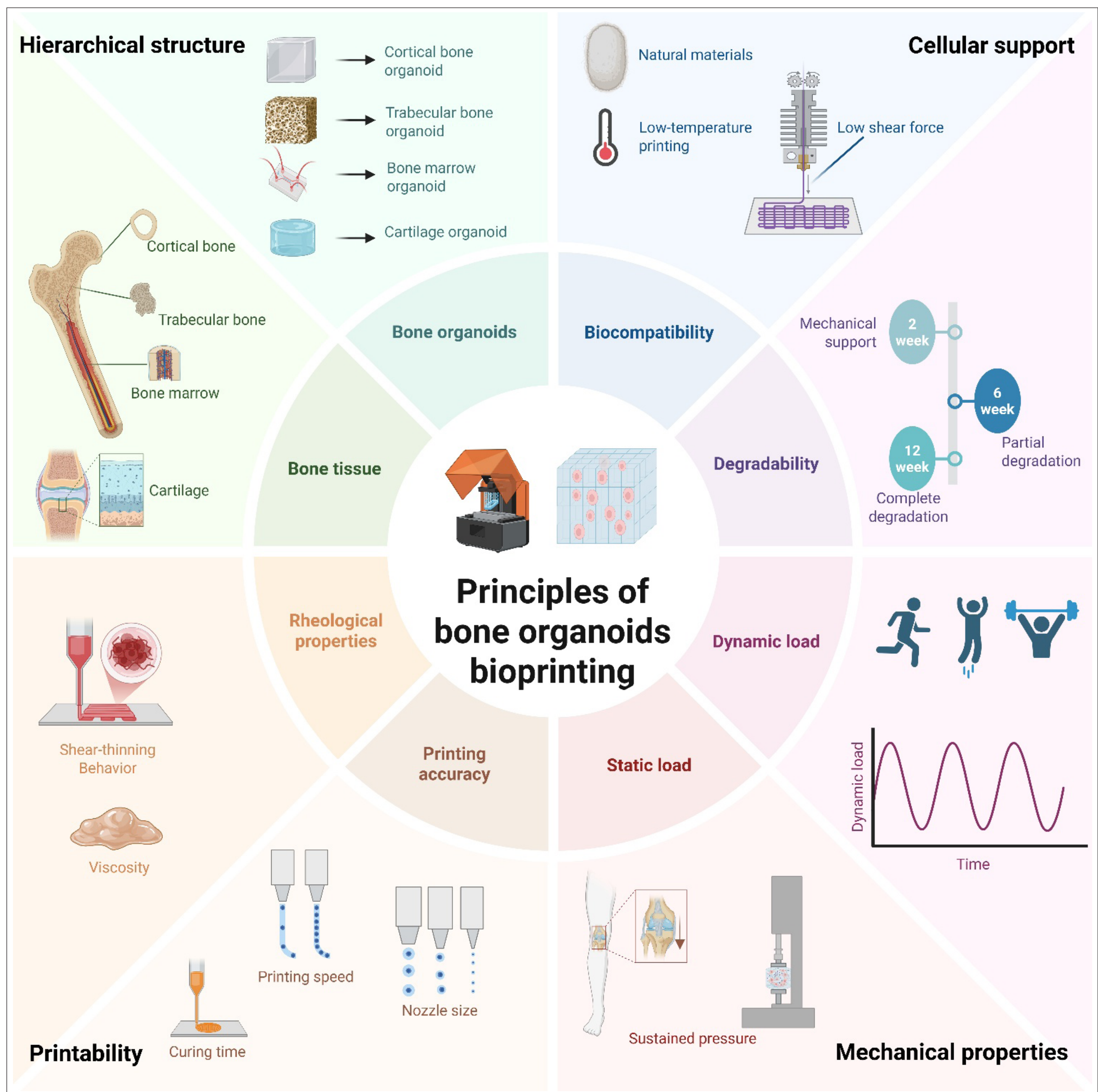

Figure 2. Basic principles of bone organoid bioprinting. This figure illustrates the key principles in designing and constructing bone organoids, focusing on the hierarchical structure, cellular support, printability, and mechanical properties required for bone organoids. Schematic diagram created by the authors.

stiffness, high elasticity, and compressive cushioning.[74] To mimic this property, hydrogel-like materials with excellent hydration and moderate modulus of elasticity are often used. These materials not only mimic the mechanical properties of cartilage but also provide a suitable environment for cell growth to support chondrocyte attachment, proliferation, and differentiation. Due to the relatively simple structure of cartilage tissue and the absence of a vascular network, the selected scaffold materials must mimic the hydration and flexibility of cartilage tissue to ensure that the constructed cartilage can effectively withstand mechanical loads and provide the required cushioning function.[75,76] At the same time, the scaffold's degradation rate must align with the growth of cartilage tissue to maintain the stability of the cartilage layer.[77,78] The connection between cartilage and bone matrix is an essential part in the design and formation

of bone organoids due to their distinct functional and structural properties. Achieving a smooth transition between these tissues in 3D scaffolds will directly affect organoid efficacy. Microfluidic printing technology enables precise gradient control at this interface through regulated inlet flow rates and ink ratios of the microfluidic mixer. By adjusting the gradient parameters according to the structural characteristics and physiological requirements, this technique facilitates the formation of a natural and stable connection between cartilage and bone matrix.[79]

The bone marrow cavity is an important part of bone tissue, containing hematopoietic stem cells, adipocytes, and BMSCs, and playing a key role in bone repair, immune response, and blood circulation.[80] Although fully immunocompetent bone marrow organoids have yet to be realized, the simulation of the medullary cavity, especially in terms of vascularization and intercellular communication, is still an important part of bone organoid construction.[81] 3D bioprinting technology can precisely control the pore structure of scaffolds to create a microenvironment suitable for the growth of bone marrow cavity cells. By using highly porous materials that mimic the spatial characteristics of bone marrow and incorporating materials such as hydrogels and synthetic polymers, the strength and biocompatibility needs of the bone marrow cavity can be met.[82] During printing, bioinks containing hematopoietic stem cells and BMSCs can be printed directly into the scaffold to ensure their uniform distribution and growth. Bioprinting technology also allows for the precise construction of vascular channels to mimic blood flow. Although current technology cannot yet fully mimic the function of bone marrow fluid, the design of microchannels and capillaries establishes a foundational framework for future bone marrow organoid construction. With ongoing technological advances, the integration of microfluidics, immune cells, and multiple types of stem cells holds promise for creating more complex bone marrow function simulations, thereby advancing the construction of bone marrow organoids.

3.1.2. Cellular support

The cell-supportive nature of bioink is reflected not only in its high biocompatibility but also in its ability to degrade harmlessly once the bone organoid has matured.[7,83] First, the chemical composition of the material needs to be compatible with the biological environment to avoid cytotoxicity or inflammatory reactions. Natural polymers like alginate and gelatin are often widely used in 3D bioprinting due to their good biocompatibility. In contrast, synthetic polymers can be chemically modified to improve their hydrogel properties, making them more adaptable to the cell growth environment.[45] In addition, the pH, ionic concentration, and osmotic pressure of bioinks must closely match the physiological environment to ensure cell viability post-printing.[84] The choice of printing method can also impact cell survival. High temperatures during printing can damage cells; therefore, most bioinks require printing at lower temperatures to avoid thermal damage (Figure 2). In particular, extrusion printing technology requires the printing temperature to be maintained below 40°C to avoid overheating-induced cell damage.[85] Other printing methods, such as inkjet, typically operate at lower temperatures, thereby reducing the thermal stress on cells. At the same time, the effect of shear forces may lead to mechanical damage to cells, especially at higher print speeds. Excessive print speeds increase the shear force during material flow, causing stress on the cells. To reduce the shear stress, the print speed should be appropriately reduced, and parameters such as print path and layer thickness must be controlled.[25] By optimizing these variables, it is possible to reduce cell damage during printing and improve cell survival.

The degradation properties of scaffolds directly affect cell differentiation and bone matrix formation. In the early stage of stem cell or precursor cell proliferation, scaffolds must maintain structural stability and provide sufficient support. At this stage, a slower degradation rate is essential to withstand cellular expansion and provide structural support for the new tissue. When cells begin to differentiate and form the initial tissue structure, the degradation rate of the scaffold can be gradually accelerated to accommodate tissue growth. The degradation products at this stage can provide nutrients to the cells while promoting cell differentiation.[51] During the final maturation stage of the bone organoids, the scaffold should gradually undergo complete and harmless degradation to allow the newly formed tissue to stabilize without interference from residual scaffold material.[86] The rate of scaffold degradation can be controlled by adjusting the cross-linking density of the material as well as the printing parameters. Specifically, increasing the material's cross-linking density, layer thickness, and reducing porosity will reduce the scaffold's degradation rate. In contrast, decreasing the cross-linking density, reducing layer thickness, and increasing porosity will accelerate the degradation process.

3.1.3. Printability and mechanical properties of bioinks

Rheological properties are the core parameters for evaluating the printability of bioinks, primarily encompassing viscosity, shear force, and elastic modulus. Viscosity determines the bioink's ability to flow smoothly and maintain the intended structure integrity during the printing process.[87] In 3D bioprinting, the viscosity of

bioink must be balanced within a certain range. When the viscosity is too low, the bioink may flow excessively, failing to maintain the predetermined shape during the printing process and potentially causing undesirable cell displacement or diffusion, which can impair cell localization and proliferation.[88] Another rheological property associated with bioinks is shear thinning, where inks typically experience high shear rates during 3D printing, especially at the nozzle. Bioinks exhibiting good shear thinning demonstrate a reduction in viscosity when subjected to an external force, allowing smooth extrusion through the nozzle and preventing clogging caused by high viscosity.[89–91] This property is particularly beneficial for detailed or high-precision printing, as it enhances print accuracy and structural stability (Figure 2). In addition, another important effect of shear thinning is the rapid recovery of viscosity after extrusion. Once the shear force is removed post-printing, the bioink's viscosity increases promptly, allowing the structure to stabilize quickly and preventing deformation of the structure from affecting the print result.

The printing accuracy and resolution of bioinks directly affect the accuracy of cell and tissue structures generated during 3D bioprinting. During the printing process, the bioink must cure promptly after deposition to form the desired structural morphology.[13,92] If curing occurs too slowly, the printed structure may become unstable; conversely, overly rapid curing can cause nozzle clogging or uneven hardening. Therefore, bioinks require an optimal cure time and hardening rate to ensure that each layer quickly forms a stable structure during the printing process, while maintaining a certain degree of fluidity to ensure accurate printing. Nozzle size and layer thickness during printing directly affect the resolution of the print.[93] Smaller nozzle sizes enable finer structures and higher resolution, but also require lower viscosity and good shear thinning of the ink to avoid clogging. Printing thinner layers enhances detail resolution but increases the stability requirements of the printing process[94,95] (Figure 2). Therefore, controlling the nozzle size and print layer thickness is critical for printing accuracy and resolution. In addition, print speed also affects print resolution. Excessively high speeds may lead to unstable ink flow, compromising print accuracy and detail fidelity. Therefore, appropriate print speeds and optimized print path strategies can ensure accurate printing at high resolutions while maintaining the stability of the printing process.

3.2. Construction process of bone organoids

3.2.1. Cell attachment and proliferation

Appropriate surface roughness has been shown to enhance cell attachment area by improving the contact between the cells and the scaffold, thereby promoting stable cell attachment.[96,97] When the surface of the scaffold is relatively smooth, it may be difficult for cells to form a stable attachment to its surface, while a scaffold with a rough surface can provide more attachment points for the cells (Figure 3). Furthermore, scaffold hydrophilicity is a critical factor affecting attachment; more hydrophilic surfaces can improve cell affinity and facilitate binding between the cell membrane and the scaffold surface.[98] Once stable attachment is established, the cells rapidly enter the proliferation stage and form a preliminary cell population. The porosity, pore size, and distribution of the scaffold directly influence the available space and growth environment for cell proliferation. Larger pores and higher porosity can provide sufficient growth space for cells to expand in a 3D environment.[99] Appropriate pore size also improves gas exchange, nutrient supply, and metabolic waste removal, creating a microenvironment favorable for cell proliferation. For example, micro- or nanopore structures can increase the attachment area of cells, thereby promoting stable cell proliferation.[82] In addition, the connectivity of the scaffold pores is also crucial for the migration and expansion of cells, enabling uniform cell distribution throughout the scaffold's 3D space. In addition, the mechanical properties of the scaffold also play a key role in cell proliferation. Scaffolds with suitable stiffness can highly simulate the mechanical environment of native bone tissue, thereby promoting cell growth.[100] Conversely, scaffolds with insufficient stiffness inhibit cell proliferation, as cells receive inadequate mechanical stimulation, leading to reduced proliferation rates.

3.2.2. Cell differentiation and bone matrix formation

Osteogenic differentiation in bone organoid construction typically involves key signaling pathways, including Wnt/β-catenin signaling, BMPs/transforming growth factor-β signaling, and others. These pathways promote stem cell differentiation into osteoblasts and stimulate the synthesis and mineralization of the bone matrix by activating osteogenesis-related gene expression.[101] To effectively regulate these signaling pathways, researchers can directly add factors such as BMP and Wnt3a into bioinks, which are co-printed alongside cells and scaffold materials (Figure 3). The factors interact with the cells to promote cell differentiation and tissue formation. Furthermore, microfluidic technology can be utilized to introduce microfluidic channels during the printing process to precisely deliver cytokines to specific locations, ensuring their uniform distribution and optimal stimulation of cells. Microenvironmental regulation is also an important factor affecting cell differentiation. Maintaining a low oxygen environment and physiological pH supports osteogenic differentiation.[102] Optimizing these microenvironmental

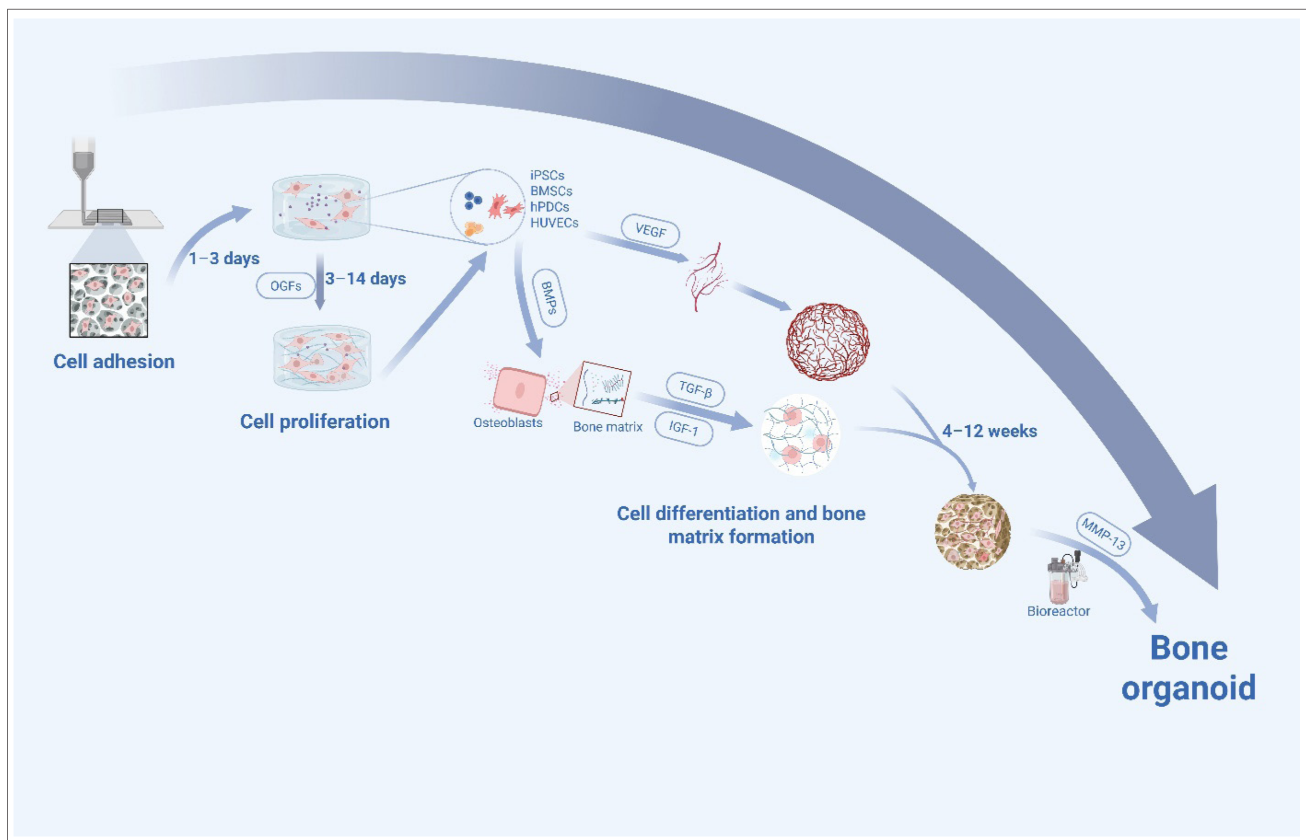

Figure 3. Overview of the bone organoid construction process Cells first adhere to the three-dimensional bioprinted scaffold, where surface roughness and porosity enhance attachment and proliferation. Stem cells then differentiate into osteoblasts and produce bone matrix under stimulation from bone morphogenetic protein (BMP)-2, transforming growth factor-β (TGF-β), and insulin-like growth factor (IGF)-1. Human umbilical vein endothelial cells (HUVECs) promote vascularization in response to vascular endothelial growth factor (VEGF). After 12 weeks of in vitro culture, calcium is stably deposited, and mechanical stimulation from a bioreactor supports final organoid maturation. Schematic diagram created by the authors. Abbreviations: BMSCs, bone marrow mesenchymal stem cells; hPDCs, human periosteum-derived cells; iPSCs, induced pluripotent stem cells; MMP, matrix metalloproteinase; OGFs, osteoblast growth factors.

conditions facilitates the construction of organoids that more closely resemble bone tissue *in vivo*.

After differentiation, osteoblasts undertake the synthesis and mineralization of the bone matrix, a process crucial not only for the structural support and biological functions of bone tissue but also for the mechanical properties and repair capacity of bone organoids.[103] The primary component of the bone matrix is collagen, particularly type I collagen. During collagen secretion, osteoblasts produce collagen fibers that gradually arrange and intertwine into a mesh-like structure, forming the foundational framework of the bone matrix. Glycosaminoglycans constitute another essential component of the bone matrix, conferring elasticity and resistance to compression by interacting with water molecules.[104] During bone repair, glycosaminoglycans help maintain matrix hydration, promote mineral deposition, and support the solidity of the bone matrix. Mineralization represents the final stage of bone matrix formation, imparting stiffness and strength to the tissue. Following the secretion of collagen fibers and other matrix components, mineralization proceeds via deposition of calcium salts, which ultimately transform the matrix into hard bone tissue. Osteoblasts secrete matrix molecules containing calcium ions and phosphates, which interact with the collagen fibers in the bone matrix and gradually deposit HAP crystals, hardening the matrix.[105] Some researchers have incorporated HAP directly into 3D-printed scaffolds as a bioink component, enhancing mechanical strength while accelerating mineralization and increasing matrix hardness.[51,106] Proteins, such as osteocalcin and osteoblastin, secreted by osteoblasts, can regulate the localization and rate of calcium salt deposition. These proteins are particularly important in the early stages of bone matrix deposition, promoting stable deposition of calcium salts and ensuring a homogeneous and orderly mineralization process.[107] In addition, osteocalcin and

bone bridging proteins can be incorporated directly into the scaffold during the early stages of bone matrix formation, enabling more precise control of the process.

3.2.3. Maturation of bone organoids

Bone tissue development involves not only the synthesis and mineralization of bone matrix at the cellular level, but also the gradual acquisition of mechanical competence to bear body weight and dynamic loads. With continuous secretion and deposition by osteoblasts, HAP crystals gradually increase in size and become more evenly distributed, with a significant increase in bone matrix mineralization.[108] Eventually, the mineralized bone matrix provides strong mechanical support, effectively resisting external loads and exhibiting the biomechanical properties of native bone tissue. The mineralization and strength enhancement of the bone matrix is a highly dynamic process involving the synergistic action of osteoblasts and osteoclasts. Osteoblasts contribute to the maturation of the bone matrix by secreting a mineralized matrix, which gradually enhances its hardness and strength. In contrast, osteoclasts regulate the microstructure and mechanical properties of the bone matrix through bone remodeling, a process that removes aged and damaged parts in the bone matrix and provides new deposition space for osteoblasts. This remodeling ensures the adaptability and elasticity of bone tissue under different physiological loads. Osteoblasts are crucial in the maturation of bone organoids, significantly aiding bone matrix reconstruction and adaptation. Their role has been well established in facilitating these processes.[109] However, accurately mimicking osteoclast activity in 3D-printed bone organoids remains a significant challenge. Advances in 3D bioprinting technology are anticipated to ensure precise spatial mapping of osteoclasts within scaffolds. Leveraging high-resolution and multi-material printing capabilities, 3D printing can construct scaffolds that provide a favorable microenvironment for osteoclast growth and functionality. For example, the differentiation and function of osteoclasts can be modulated through inducible factors such as receptor activator of nuclear factor κ B ligand and macrophage colony-stimulating factor, enabling precise control over their activity during bone matrix mineralization and maturation. This dynamic regulation not only facilitates a more physiologically relevant simulation of bone remodeling but also provides a foundation for investigating the intricate cellular crosstalk involved in skeletal homeostasis and pathological bone resorption.

3.2.4. Vascularization strategies in bone organoid

With the maturation of the bone matrix, the formation of a vascular network becomes essential to sustain the viability and metabolic function of the tissue. Vascularization not only facilitates nutrient and oxygen delivery but also plays a pivotal role in maintaining cell viability, removing metabolic waste, and promoting osteogenic maturation within the organoid structure. HUVECs can be co-cultured with BMSCs by encapsulating them in biocompatible hydrogels such as gelatin methacrylate or fibrin.[110] Therefore, in the context of 3D bioprinting, both cell types can be co-printed. These hydrogels provide a favorable matrix environment for endothelial cell self-organization, enabling HUVECs to spontaneously form microvascular networks within the printed constructs.

Beyond single-cell strategies, the incorporation of microvascular fragments (MVFs) has emerged as a promising approach to accelerate vascularization. MVFs are multicellular segments derived from adipose or other vascular-rich tissues and retain intact vessel structures, including endothelial and perivascular cells.[111] When embedded in printable hydrogels and bioprinted into scaffolds, MVFs can rapidly anastomose with host vasculature post-implantation, supporting early perfusion and enhancing tissue survival. Their application has been shown to significantly improve vascular integration and bone regeneration in preclinical models.[112]

Additionally, microfluidic chips offer a complementary *in vitro* strategy for engineering vascular networks in bone organoids. Unlike traditional static co-culture systems, microfluidic platforms can simulate dynamic blood flow and shear stress within precisely patterned channels. Endothelial cells cultured within these channels align and form perfusable capillary-like structures that mimic the hierarchical vascular architecture.[113] When integrated with bioprinted constructs or used as prevascularized modules, microfluidic chips contribute to improved oxygen and nutrient diffusion and offer a controlled platform for investigating vascular–osteogenic interactions.

Beyond preformed constructs, host vessel ingrowth after implantation is also a widely adopted strategy. Instead of relying solely on *in vitro* prevascularized constructs, this approach utilizes the body's intrinsic angiogenic potential to support the gradual infiltration of host-derived blood vessels into the implanted scaffold. After transplantation, the scaffold serves as a physical guide and biochemical microenvironment that encourages endothelial migration, capillary sprouting, and anastomosis with the native vasculature.[114] To facilitate this process, 3D bioprinting can be used to precisely design scaffolds with optimized pore size, interconnectivity, and spatial architecture, which are essential for vessel ingrowth. Unlike artificial vascular networks formed *in vitro*, host-derived vasculature tends to follow physiologically relevant spatial distributions and supports long-term remodeling and integration with

surrounding bone tissue. This strategy is particularly valuable in large bone defects or critical-sized lesions, where robust vascular support is essential for sustained tissue survival, osteogenesis, and integration.[115] Therefore, harnessing host vascular ingrowth through the design of pro-angiogenic 3D-printed scaffolds offers a biologically compatible and translationally promising route toward functional bone organoid development.

3.2.5. Physiological load simulation and functional recovery of bone organoids

Bone tissue is a dynamic structure whose function and morphology are continuously affected by physiological loads. To construct bone organoids with practical clinical relevance, it is essential to consider the role of physiological loads and promote the functional recovery of bone organoids through appropriate mechanical stimulation.[116] Physiological loads include mechanical stimuli such as pressure, tension, and bending that bone tissues experience *in vivo*. These external forces not only affect the physiological adaptation of bone tissues but also critically influence their mechanical properties and repair processes. Mechanical stimulation plays a crucial role in activating the Wnt signaling pathway within osteoblasts, thereby facilitating their proliferation and differentiation. This process not only enhances cellular activity but also strengthens the mineralization of the ECM produced by osteoblasts, ultimately contributing to bone tissue formation and remodeling.[117] In addition, appropriate mechanical loading can regulate the deposition pattern of HAP, promoting its uniform distribution within bone organoids. This uniform mineralization prevents brittleness caused by uneven HAP deposition and improves the overall mechanical integrity of the construct. To replicate the mechanical stimuli endured by bone tissues *in vivo*, a bioreactor can be used in experimental studies to apply periodic compressive loads or tensile loads to the printed scaffolds. Such mechanical conditioning enables cells to perceive and adapt to the external mechanical environment, further optimizing the formation and functional integration of the bone organoids.

4. Challenges

4.1. Challenges in simulating bone tissue structure

The construction of bone organoids aims to mimic the structure and function of natural bone tissues to meet the needs of bone tissue regeneration, disease research, and drug screening. However, due to the physiological complexity of bone tissues, current research emphasizes the creation of functionally specific bone organoids, while complex bone organoids with complete physiological functions have not yet been realized.[101] Current bone organoids are usually constructed for a single tissue, such as cortical bone, cancellous bone, or cartilage, and the integration of multiple tissue types has not yet been achieved. Cortical bone organoids are mostly used for mechanical studies, cancellous bone organoids are more inclined to mimic vascularized microenvironments, and cartilage organoids are mainly used for the study of cartilage injury and degenerative diseases.[118] Due to the significant differences in the structure and cellular composition of these tissues, it remains a technical challenge to integrate different tissues into the same bone organoid model. Future studies could employ bioinks with partitioning properties to accurately reproduce the physical properties of different tissues within a single construct.[79] For example, mineralized biomaterials could be used to print cortical bone with high mechanical strength while combining cell- and growth factor-rich hydrogels to form cancellous bone and bone marrow cavities. In addition, the key to multi-tissue integration lies in the optimization of the transition region between different materials. Establishing a reasonable mechanical gradient between cortical and cancellous bone is essential to improve the overall mechanical stability and physiological adaptability of the composite bone organoid.

The natural bone matrix is fundamentally composed of a composite microenvironment formed by the interweaving of collagen fibers and HAP nanocrystals. However, existing extrusion and light-curing 3D bioprinting technologies, which typically have a resolution of tens to hundreds of microns, fall short of replicating the nanoscale precision required to mimic collagen fiber arrangement.[119] In addition, mineralization of natural bone tissues is regulated by biochemical signals, and existing bioprinting technologies struggle to synchronously guide mineralization during fabrication. Even when mineralization is induced by subsequent culturing, achieving uniform and hierarchical mineral deposition remains challenging. Electrospinning offers a promising complementary approach by utilizing high voltage to generate electrostatic forces that draw polymer solutions or melts into ultrafine fibers, which are then deposited into nanoscale fibrous membranes. The technique can fabricate continuous fibers ranging from nanometers to microns in diameter. Looking ahead, the integration of alternating superposition of electrostatic spinning layers and 3D printing layers holds potential for hierarchical construction, from nanoscale collagen fiber networks to micrometer-scale scaffolds, providing a more physiologically structured support system for bone organoids.[120] Additionally, employing 3D printing to fill electrospun scaffolds could improve cell arrangement and tissue integration efficiency. For instance, 3D printing with bioinks enriched with BMSCs or hPDCs between the nanofiber layers formed by electrostatic

spinning can enhance cell adhesion and osteogenic differentiation potential.

4.2. Vascularization challenges in bone organoid construction

Vascularization of bone tissue is crucial for nutrient transport, oxygen exchange, and waste removal, playing a central role in bone development, regeneration, and homeostasis.[121] However, current strategies for vascularizing bone organoids still face significant technical and biological limitations.

One major challenge is the difficulty in constructing complex microvascular networks using existing 3D bioprinting technologies.[122] Although techniques such as coaxial extrusion and sacrificial molding have shown promise, achieving stable, perfusable lumens with physiologically relevant diameters remains difficult. Printed vascular channels often collapse due to insufficient mechanical support or become occluded by uncontrolled cell proliferation. Moreover, vascular endothelial cells such as HUVECs are highly sensitive to hypoxic conditions, and their survival and function decline in poorly perfused or static environments.[123] Maintaining their viability within dense 3D structures remains a critical bottleneck. In addition, it is difficult to achieve an optimal balance between the biocompatibility and mechanical strength of vascular scaffolds. Materials that support cell adhesion and angiogenesis often lack the stiffness required to preserve lumen structure under dynamic flow conditions.

Another limitation is the lack of functional integration between vascular and osteogenic components. In many constructs, blood vessels form structurally but fail to interact efficiently with bone-forming cells.[124] This lack of paracrine signaling and mechanical communication limits bone matrix maturation and remodeling. Furthermore, reproducing the spatial and temporal dynamics of the native bone microenvironment—including gradients of oxygen, nutrients, and shear stress —remains difficult in static culture systems.

The cell source and heterogeneity of endothelial cells also present challenges. Commonly used HUVECs may not fully represent bone microvascular endothelial phenotypes, and their limited lifespan and variability reduce reproducibility.[125] Meanwhile, achieving vascular anastomosis with host vessels upon implantation remains unpredictable, and inadequate integration can result in central necrosis of the engineered tissue.

Overcoming these challenges will require the integration of advanced bioprinting strategies, dynamic perfusion bioreactors, tissue-specific endothelial cell sources, and real-time functional imaging. Addressing the vascularization bottleneck is essential to developing clinically relevant and physiologically functional bone organoids.

4.3. Inadequate dynamic modeling of the physiological environment

Bone tissues continuously experience complex mechanical stimuli *in vivo*, including fluid shear, cyclic compressive stress, and tensile torsion. However, existing *in vitro* culture environments are mostly static. Even bioreactors currently in use typically provide only a single mechanical stimulus, making it difficult to mimic the real mechanical environment *in vivo*, leading to significant deficiencies in functional maturity and tissue integration of printed bone organoids.[116] For example, mesenchymal fluid shear regulates osteoblast differentiation, and static culture systems fail to provide this critical signal, limiting the formation of mineralized matrix. In addition, conventional bioreactors struggle to precisely regulate mechanical stimuli and nutrient gradients, which affect the growth and functionalization of bone organoids.[126]

These shortcomings can be remedied in the future by developing an intelligent dynamic culture system. In combination with microfluidic mechanical stimulation devices, multi-dimensional mechanical signals can be provided within 3D-bioprinted tissues to enhance cell–matrix interactions. At the same time, the medium flow pattern can be optimized to establish a nutrient gradient closer to the physiological environment and to improve the maturation of bone organoids.[127] Further directions include intelligent bioreactors, combining sensing technology and artificial intelligence algorithms for real-time monitoring, and dynamic regulation of parameters such as mechanical stimulation, pH, and oxygen concentration. Such advancements will enhance the quality of *in vitro* culture of bone organoids and provide a foundation for future clinical translation.

4.4. Limitations in the application of biomaterials

There are still many limitations in the use of current materials for bone organoid bioprinting. Firstly, it is difficult to balance mechanical properties and biocompatibility. Most natural hydrogels have good biocompatibility but low mechanical strength, while synthetic polymers have high mechanical strength but lack cell adhesion sites, which affects cell proliferation and differentiation. Secondly, the degradation rate of materials is difficult to align with bone tissue regeneration. Certain materials degrade too fast, which may lead to insufficient support, while others degrade too slowly, which may increase the probability of bacterial and fungal contamination and lead to experimental failure. In addition, material preparation and printing compatibility issues also affect the efficiency and precision

of 3D bioprinting, and high-performance biomaterials often affect the printing effect due to poor rheology, nozzle clogging, or uneven curing. Incorporating both natural and synthetic polymers into bioink formulations offers a promising strategy to simultaneously enhance scaffold stability and cytocompatibility. However, achieving a homogeneous and structurally robust composite requires precise optimization of surface modifications or synergistic cross-linking techniques. Without such refinements, phase separation may occur, compromising the mechanical properties and long-term stability of the scaffold, which is crucial for biomedical applications. Meanwhile, combining natural materials with synthetic materials often involves multi-step preparation such as freeze-drying, solvent evaporation, and hot pressing. Complex fabrication processes may lead to large differences in compositional homogeneity, mechanical properties, and bioactivity of bioinks, resulting in poor batch stability and reproducibility, affecting their clinical translation.

Temperature-responsive hydrogels and photoresponsive hydrogels exhibit fluidity during printing, facilitating printing operations. After printing, they rapidly transition back to a gel state, significantly improving the stability of the constructed structure. This feature not only ensures the mechanical strength of the material for tissue construction but also effectively prevents cell damage. Given these advantages, temperature-controlled responsive hydrogels are poised to become a primary focus in bioink research. Regarding the construction of composite bioinks, it is necessary to establish a database of the optimal ratios of different natural polymers and synthetic polymers, and establish standardized printing parameters (e.g., nozzle temperature, pressure, printing speed, etc.) for different material systems. Such standardization will reduce inter-experimental variability, thereby improving reproducibility and accelerating the clinical translation of 3D-bioprinted bone organoids.

5. Conclusion

Three-dimensional bioprinting represents a groundbreaking advancement in bone organoids, enabling precise fabrication of biomimetic constructs with tailored biological and mechanical properties. Through innovative bioink formulations and advanced printing techniques, researchers have achieved remarkable progress in replicating the hierarchical structure of bone. The integration of vascular networks via co-printing endothelial cells and the use of dynamic bioreactor systems has further enhanced organoid functionality, bringing us closer to clinically viable solutions for bone regeneration.

Despite these achievements, significant challenges remain. The limited scalability of current bioprinting methods hinders large-scale clinical application, while long-term stability issues related to immune response and scaffold degradation require further investigation. Additionally, fully recapitulating the complex interplay between bone, cartilage, and vascular tissues within a single construct remains technically demanding. Addressing these limitations will be crucial for translating laboratory successes into therapeutic realities.

Looking ahead, future research should prioritize the development of smart, stimuli-responsive bioinks capable of controlled growth factor release, as well as the incorporation of multi-omics data to optimize cellular composition. Combining 3D bioprinting with artificial intelligence-driven design and large-animal validation studies will accelerate progress toward personalized bone repair strategies. As this field evolves, the synergy between engineering innovation and biological insight promises to revolutionize regenerative medicine, offering new hope for patients with critical bone defects.

List of abbreviations

Abbreviation	Full term
3D	three-dimensional
BMP	bone morphogenetic protein
BMSCs	bone marrow mesenchymal stem cells
DLP	digital light processing
ECM	extracellular matrix
HAP	hydroxyapatite
hPDCs	human periosteum-derived cells
HUVECs	human umbilical vein endothelial cells
iPSCs	induced pluripotent stem cells
MVFs	microvascular fragments
SLA	stereolithography

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Conflict of interest

The authors declare no conflict of interest.

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Further disclosure

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