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REVIEW

Advances and Challenges in 3D Bioprinting of Cartilage Organoids: From Material Innovation to Functional Regeneration

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Abstract: Articular cartilage injury and degenerative disorders are major contributors to joint dysfunction. Because cartilage is avascular and aneural, its intrinsic healing capacity is extremely limited, which continues to present a major clinical challenge. Cartilage organoids, defined as three-dimensional constructs that recapitulate key structural, cellular, and functional features of native cartilage tissue, including cell–cell and cell–matrix interactions, zonal organization, and responsiveness to biochemical and mechanical cues, have emerged as promising platforms for cartilage regeneration and disease research. In this context, three-dimensional (3D) bioprinting offers a powerful top-down strategy for fabricating such organoids with high spatial precision, complementing conventional bottom-up self-assembly approaches. This capability has opened new opportunities for the generation of cartilage constructs with complex architectures and biomimetic functions, highlighting their potential in personalized therapy, disease modeling, and regenerative medicine. This review provides a comprehensive overview of recent progress in 3D bioprinting for cartilage organoid engineering. First, it summarizes advances in bioink design, ranging from natural and synthetic hydrogels to composite and reinforced systems, such as the emerging attapulgite-polyvinyl alcohol platform, as well as stimulus-responsive smart materials. These materials are being developed to better replicate the biochemical and mechanical properties of the native extracellular matrix while maintaining suitable printability. Second, the review discusses the principles, optimization strategies, and application characteristics of major bioprinting techniques, including extrusion-based, photocuring-based, inkjet, and microfluidic bioprinting, with particular emphasis on balancing printing fidelity, structural complexity, and cell viability. In addition, it examines key strategies for promoting functional maturation and in vivo integration of cartilage organoids, including coculture systems, direct vascularization approaches, spatiotemporally controlled delivery of growth factors, dynamic mechanical stimulation, and emerging osteoimmunomodulatory interventions such as macrophage polarization and neutrophil extracellular trap clearance. Despite substantial advances, several critical challenges remain, including limited biomimetic accuracy in hierarchical architecture, instability of long-term cell phenotype, difficulties in vascularizing large-scale constructs, and the absence of standardized criteria for clinical translation. By identifying these bottlenecks and outlining future directions, including the development of 4D bioprinting materials and the integration of organ-on-a-chip systems with artificial intelligence-based optimization, this review aims to support the evolution of cartilage organoids from structural mimics toward truly functional regenerative constructs, ultimately facilitating their translation into clinically applicable therapies.

Keywords: cartilage organoids, three-dimensional bioprinting, bioink, tissue engineering, functionalization, joint repair

Introduction

Articular cartilage, as a highly specialized connective tissue, plays a crucial role in reducing joint friction and buffering mechanical loads. However, due to its lack of direct blood supply, lymphatic circulation, and innervation, its intrinsic repair capacity is extremely limited once injured (eg, sports trauma, osteoarthritis), often leading to progressive degeneration. Globally, osteoarthritis affects over 500 million people, imposing a heavy socio-economic burden.

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Traditional clinical repair strategies, such as microfracture, autologous chondrocyte implantation (ACI), and matrix-induced autologous chondrocyte implantation (MACI), while showing some efficacy, commonly suffer from issues like donor site morbidity, the formation of fibrocartilage rather than hyaline cartilage in the repaired tissue, suboptimal mechanical properties, and poor integration.[1]

Native articular cartilage exhibits a highly organized zonal structure from the superficial to deep zones, characterized by gradient distributions of cell morphology (flattened chondrocytes in superficial zone vs. rounded in deep zone), matrix composition (higher proteoglycan content in deep zone), collagen fiber orientation (parallel to surface in superficial zone vs. perpendicular in deep zone), and mechanical properties (increasing compressive modulus from surface to subchondral bone). This zonal complexity necessitates gradient bioink strategies that can recapitulate such hierarchical organization. In recent years, tissue engineering centered on 3D bioprinting technology has provided a novel solution for cartilage regeneration. This technology can, based on digital models, precisely position "bioinks" containing living cells, biomaterials, and bioactive factors in a layer-by-layer deposition manner, constructing 3D cartilage organoids with anatomically matched shapes and biomimetic microstructures. Compared to traditional methods, the unique advantage of 3D bioprinting lies in its exceptional spatial manipulation capability, enabling the simulation of native cartilage's extracellular matrix (ECM) gradients, cell alignment, and heterogeneous component distribution from the superficial to deep zones, which is fundamental for achieving functional regeneration.[2] Table 1, Figures 1 and 2

Currently, research on bioprinting cartilage organoids is transitioning from "structural imitation" to a new stage of "functional biomimicry." Researchers are not only focused on printing morphologically realistic scaffolds but are also dedicated to endowing them with long-term stable chondrocyte phenotype, suitable mechanical properties, and functional integration capability with host tissue. Despite notable progress, key bottlenecks hindering clinical translation remain, including how to balance the printability and biocompatibility of bioinks, how to ensure cell survival and uniform differentiation within large-scale organoids, how to construct vascularized interfaces promoting integration, and how to establish standardized quality assessment systems. This review aims to systematically outline the latest breakthroughs in materials, technologies, and functionalization strategies in this field, and to delve into current challenges and future development directions, providing a comprehensive reference for cartilage regenerative medicine research and application.[3] Unlike conventional tissue-engineered constructs that primarily serve as scaffolds for cell delivery, cartilage organoids are defined as three-dimensional, self-organized cellular aggregates that recapitulate key structural, cellular,

Table 1 Comparison of Scaffold-Free, Scaffold-Based, and Bioprinted Strategies for Cartilage Organoid Preparation

Preparation Strategy	Main Characteristics	Advantages	Disadvantages	Representative Applications
Scaffold-free (self-assembly)	Relies on the intrinsic self-organization and aggregation capacity of cells to form spheroids or microtissues without exogenous supporting materials	Avoids foreign scaffold interference; promotes natural cell-cell interaction; beneficial for studying self-organization and cell-driven tissue formation	Limited control over construct size and geometry; weak mechanical stability; difficult to reproduce complex anatomical structures	Cell spheroids, microtissue formation, early-stage cartilage organoid models, disease modeling
Scaffold-based (traditional)	Uses prefabricated porous or hydrogel scaffolds as structural templates to support cell attachment, proliferation, and matrix deposition	Provides initial mechanical support; facilitates shape maintenance; relatively mature and easy to implement	Limited spatial control of cells and materials; difficult to mimic zonal heterogeneity of native cartilage; possible scaffold-related interference with cell behavior	Cartilage tissue engineering, defect filling, in vitro chondrogenesis studies
Bioprinted	Deposits cells and biomaterials layer by layer in a predefined manner to generate constructs with controlled architecture and composition	Enables patient-specific design; high spatial control of cells/materials; suitable for reproducing zonal organization, gradient properties, and complex structures	Printing parameters may affect cell viability; bioink design remains challenging; post-printing maturation and long-term functional stability still need improvement	Personalized cartilage constructs, osteochondral models, regenerative medicine, drug screening, disease modeling

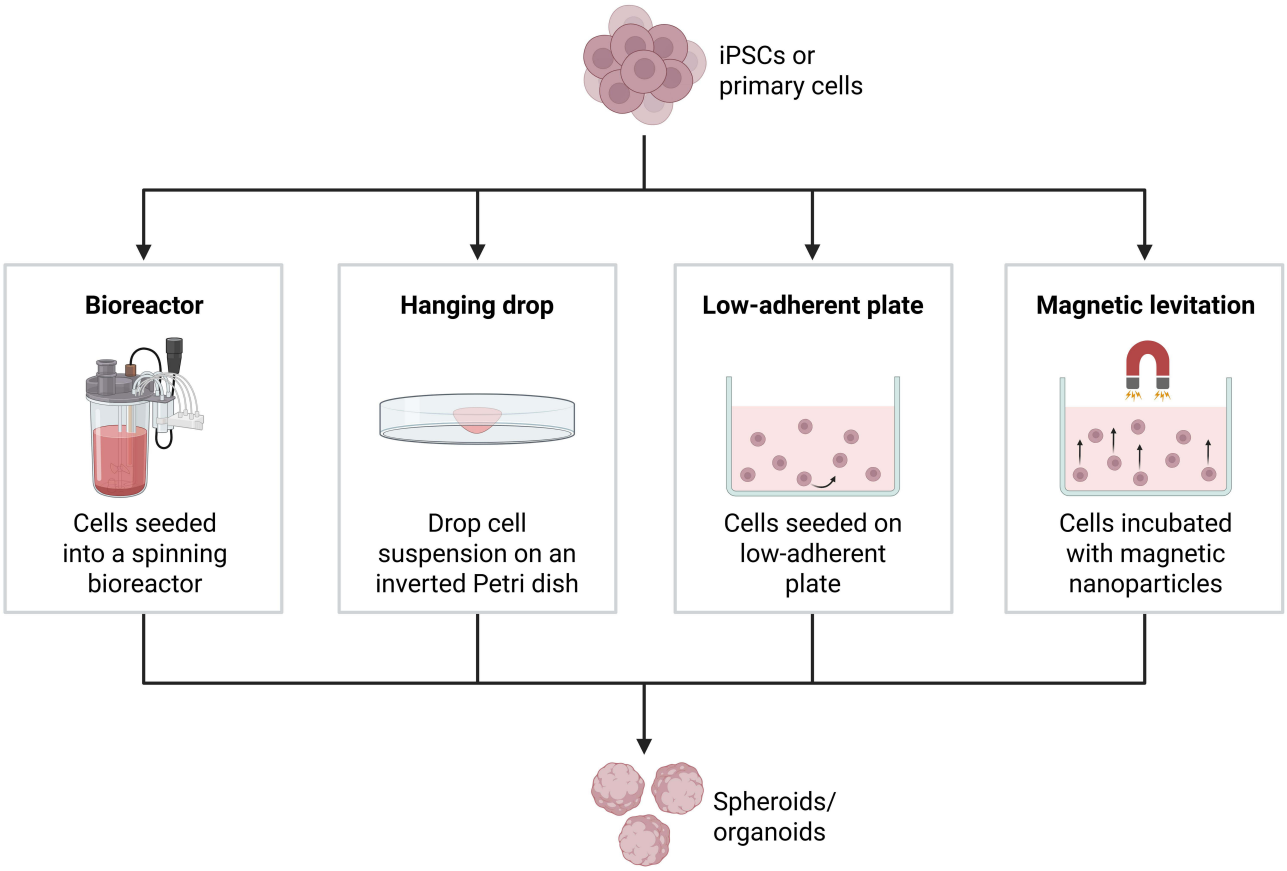

Figure 1 Overview of four classic methods for organoid/spheroid production. This figure illustrates the workflows for generating spheroids or organoids from induced pluripotent stem cells (iPSCs) or primary cells via four approaches: bioreactor, hanging drop, low-adherent plate culture, and magnetic levitation.

and functional features of native cartilage tissue, including cell–cell and cell–matrix interactions, zonal organization, and responsiveness to biochemical and mechanical cues.

While several bioprinted cartilage constructs have entered preclinical large-animal studies and a few have progressed to early-phase clinical trials (eg, for auricular and nasal cartilage reconstruction), no 3D-bioprinted articular cartilage organoid product has yet received regulatory approval (eg, FDA or EMA) for routine clinical use, highlighting the significant gap between research advances and clinical translation.

Unlike conventional tissue-engineered constructs, which are often designed mainly as scaffolds for cell delivery, cartilage organoids should be understood as three-dimensional cellular systems capable of reproducing important structural, biological, and functional characteristics of native cartilage, including cell–cell interactions, cell-matrix communication, and tissue-specific organization. In discussing this concept, it is useful to distinguish between two related but fundamentally different strategies: bottom-up self-assembly, in which organoid formation arises from the intrinsic capacity of cells to organize themselves, and top-down bioprinting, in which cells and biomaterials are deposited in a predefined, layer-by-layer manner. Rather than replacing self-assembly, 3D bioprinting provides a controllable structural and material framework that can direct subsequent cellular organization, extracellular matrix deposition, and tissue maturation. Compared with traditional scaffold-based approaches, bioprinting is particularly advantageous for reproducing the spatial complexity of articular cartilage, such as zonal variation in cell phenotype, gradients in mechanical properties, and the incorporation of vascular-like channels. In this sense, bioprinting can be regarded not simply as a fabrication technique, but as a platform that defines the initial architecture and microenvironmental cues required for cells to progressively organize into functional cartilage-like tissue units.

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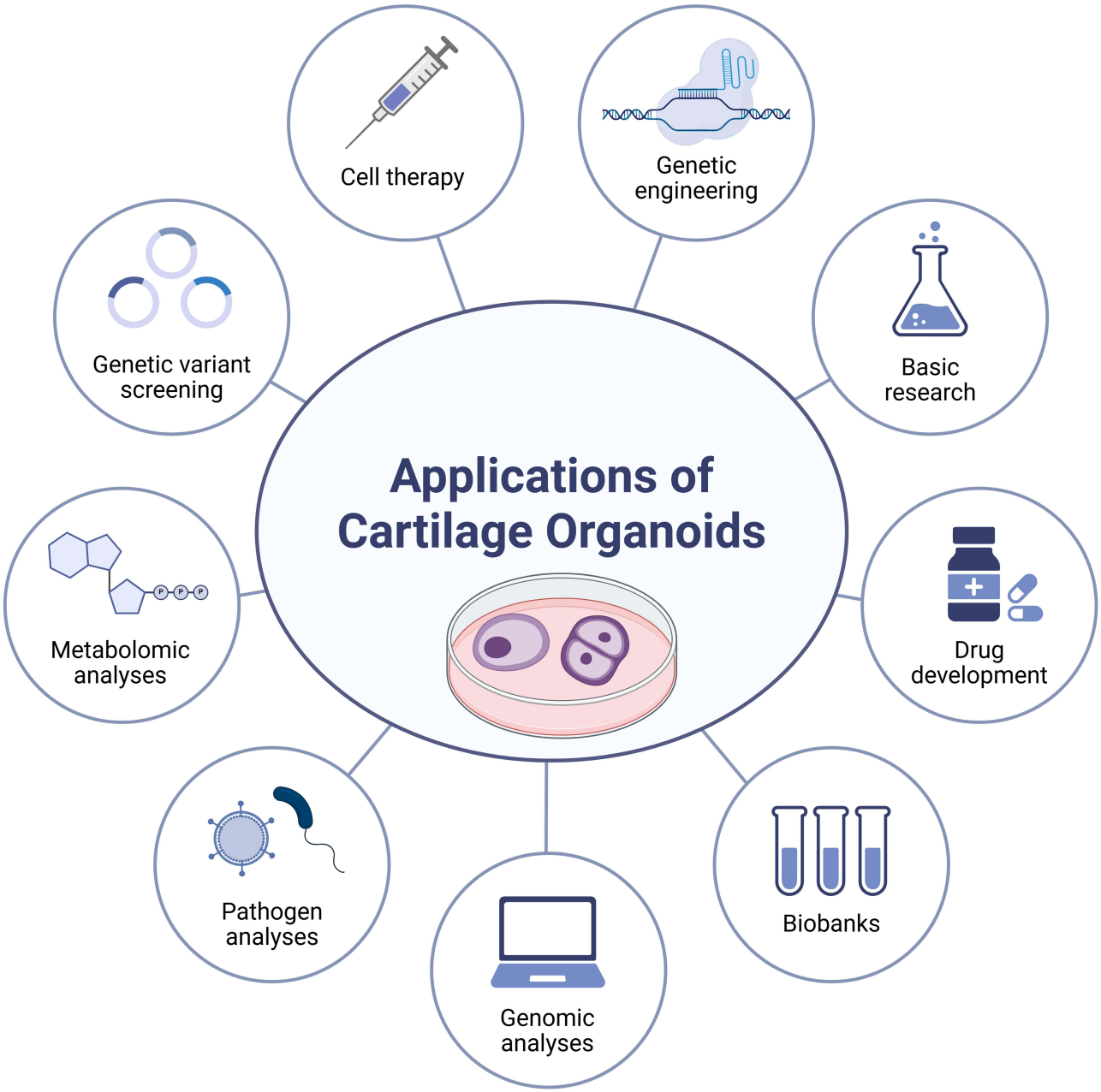

Figure 2 This figure summarizes the major applications of cartilage organoids. Cartilage organoids can be used in basic research to model cartilage development, homeostasis, and disease pathogenesis. In drug development, they serve as platforms for efficacy and toxicity screening. In combination with genetic engineering and cell-based therapies, cartilage organoids contribute to advances in precision medicine and regenerative medicine. In addition, cartilage organoids can be applied to genomic, metabolomic, and pathogen analyses for genetic variant screening and disease mechanism studies, and can be incorporated into biobanks as valuable biological resources, supporting translational research and personalized therapeutic strategies.

Bioink: The Cornerstone of Cartilage Organoid Construction

Bioink is the "living material" of 3D bioprinting, and its performance directly determines the feasibility of the printing process, the fate of encapsulated cells, and the functionality of the final construct. An ideal cartilage bioink must meet stringent criteria: excellent biocompatibility and support for cell viability, rheological properties compatible with the printing process, simulation of the biochemical and mechanical microenvironment of native cartilage ECM (Extracellular Matrix), and controllable degradation kinetics matching the tissue regeneration rate.[4] Table 2 and Figure 3

Table 2 Commonly Used Cartilage Bioink Materials and Their Characteristics Comparison

Bioink Class	Representative Examples	Main Strengths	Main Limitations	Typical Application Orientation
Natural ECM-mimetic hydrogels	GelMA, HAMA, alginate, collagen, gelatin	Good biocompatibility; supports cell encapsulation; relatively mild gelation conditions; suitable for chondrogenic microenvironment design	Usually insufficient mechanical strength when used alone; print fidelity and long-term shape retention may be limited	Basic cartilage organoid encapsulation, zonal cartilage-like microenvironment construction
Synthetic tunable hydrogels	PEGDA, PEG-based derivatives, Pluronic-based sacrificial/support inks	Highly controllable chemistry, stiffness, and degradation; good batch consistency; suitable for precise structural design	Bioinert without further modification; usually requires incorporation of adhesion motifs or bioactive components	High-resolution printing, structural support, customized patient-specific geometry
Tissue-specific dECM-containing bioinks	Cartilage dECM/GelMA, dECM/alginate, solubilized cartilage matrix blends	Provides tissue-specific biochemical cues; beneficial for phenotype maintenance and matrix deposition; improves biomimicry	Source variability, decellularization complexity, and weaker standalone mechanics; composition standardization remains difficult	Biomimetic cartilage organoids, phenotype preservation, disease modeling, regenerative constructs
Bioactive nanocomposite bioinks	GelMA/nHA, alginate/BG, nanoclay- or nanosilicate-reinforced hydrogels	Improved rheology, printability, and mechanical reinforcement; can introduce bioactive ions or osteochondral interface cues	Nanoparticle dispersion and agglomeration remain concerns; excessive loading may affect cell compatibility and extrusion behavior	Osteochondral interface engineering, gradient tissue construction, load-bearing cartilage repair
Microgel/granular bioinks	Hyaluronic acid microgels, gelatin/GelMA microgels, cartilage microtissue-supporting granular systems	Excellent shear-thinning and self-supporting behavior; improved porosity and nutrient transport; offers more space for cell remodeling and fusion	Preparation and crosslinking are more complex; mechanical uniformity and long-term integration still require optimization	Bottom-up organoid assembly, embedded printing, large constructs with enhanced cell reorganization
Dynamic/smart responsive bioinks	Supramolecular hydrogels, enzyme-sensitive hydrogels, hypoxia-mimicking hydrogels, DNA-encoded dynamic hydrogels	Dynamic remodeling, self-healing, stimulus responsiveness; better suited for regulating stem cell fate and organoid maturation microenvironment	System design is complex; reproducibility, mechanical stability, and translational standardization are still insufficient	4D bioprinting, hypoxia-regulated cartilage organoids, immunomodulatory regeneration, functionally adaptive tissue maturation

Material Classification and Performance Optimization

Hydrogels for Cartilage Tissue Engineering

Natural materials like Gelatin Methacryloyl (GelMA), Sodium Alginate, Hyaluronic Acid (HA), and decellularized cartilage extracellular matrix (dECM) are widely used due to their bioactivity, biodegradability, and cell recognition sites. GelMA, containing the RGD sequence, promotes cell adhesion and allows precise photocrosslinking, with tunable mechanical strength and degradation rate. Sodium Alginate rapidly gels via ionic crosslinking (eg, with Ca^{2+}) but lacks cell adhesion sites, requiring combination with other materials. HA, often used for cartilage tissue engineering, can be photocrosslinked to support chondrocyte signaling, though its mechanical strength is weak. dECM retains native cartilage tissue's protein composition and conformation, promoting cartilage-specific gene expression and proteoglycan deposition.[5–8] Synthetic hydrogels like Polyethylene Glycol (PEG) and its derivatives offer tunable mechanical properties but are bio-inert, so functionalization with RGD peptides or growth factors is required to guide cell behavior.[9,10] Composite strategies, including inorganic-organic composites with nanoparticles like nano-hydroxyapatite (nHA) or bioactive glass (BG), enhance compressive modulus and release pro-chondrogenic ions, while fiber-reinforced composites using electrospun synthetic fibers (eg, PCL, PLGA) mimic collagen networks, improving toughness and fatigue

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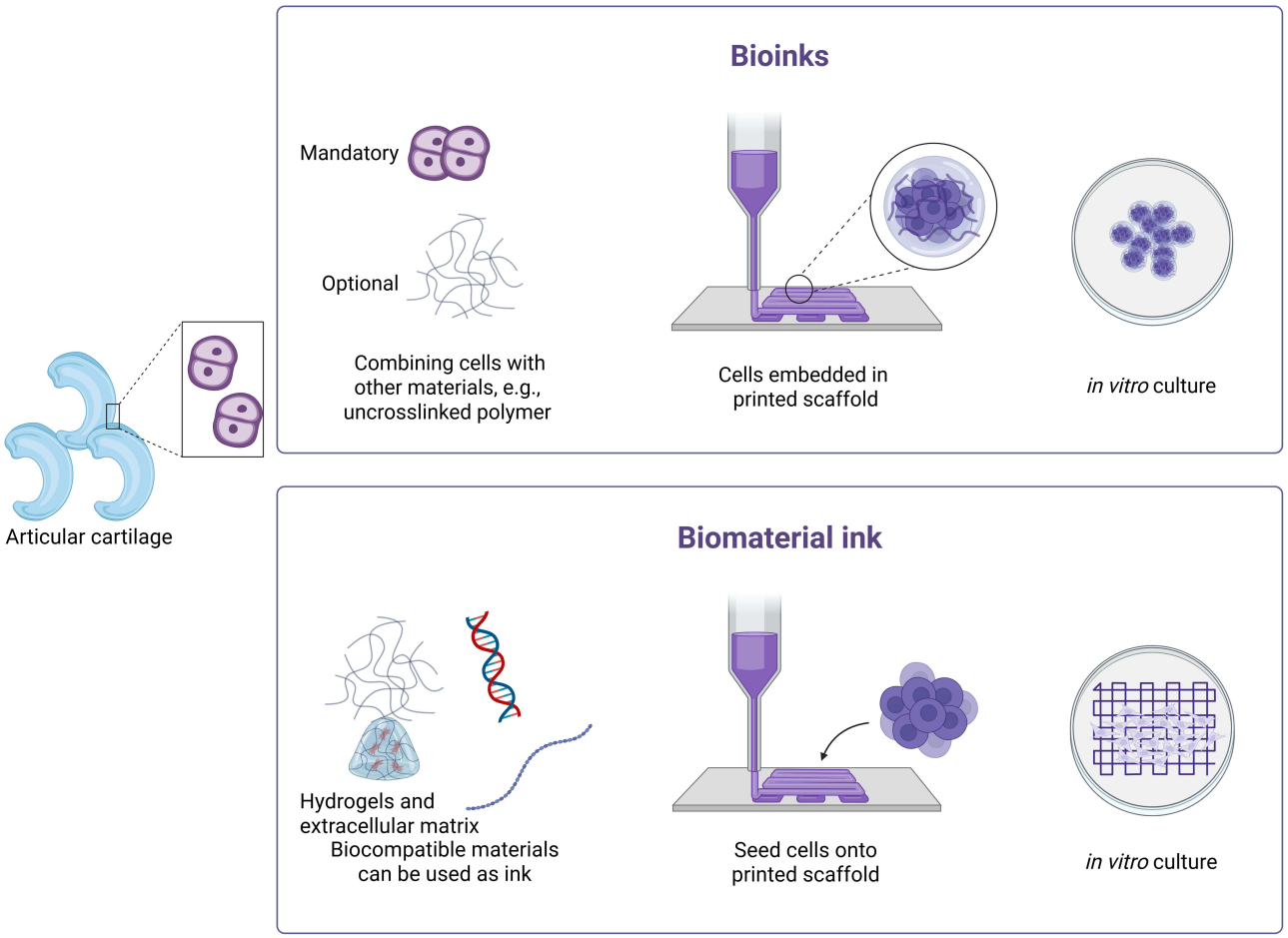

Figure 3 This schematic illustrates two strategies for integrating cartilage-derived cells or organoids with 3D bioprinting approaches. In the bioink-based strategy, cells are combined with hydrogels, extracellular matrix components, or other biocompatible materials to generate printable biomaterial inks, which are subsequently printed into scaffolds and maintained in vitro. In the biolink-based strategy, cells or organoids—optionally combined with additional materials—are directly embedded within printed scaffolds, followed by in vitro culture. These complementary approaches enable the fabrication of cartilage-mimetic constructs and provide versatile platforms for cartilage tissue engineering and regenerative applications.

resistance. Multi-component hybrids, such as GelMA-HA-PEG composites, allow fine-tuning of mechanical properties and degradation rates while maintaining cytocompatibility.[11–13]

Novel Composite Material: Attapulgite-Polyvinyl Alcohol Composite Hydrogel

Among various composite bioink explorations, the attapulgite-polyvinyl alcohol composite hydrogel, as an emerging inorganic-organic hybrid material, offers a unique and promising solution to the bottleneck problem of balancing mechanical strength, bioactivity, and printability in cartilage bioprinting.[14]

Attapulgite is a natural one-dimensional nanoscale hydrated magnesium aluminum silicate mineral. Its basic structural unit is a ribbon-like structure formed by two layers of silicon-oxygen tetrahedral sheets sandwiching a layer of magnesium (aluminum)-oxygen octahedral sheet, extending along the C-axis to form unique nanorod crystals.[15] This structure endows it with several properties highly valuable for bioprinting: Its surface contains numerous silanol (Si-OH) groups, facilitating chemical modification and providing abundant cell adhesion sites.[16] It can strongly adsorb biomolecules like proteins and growth factors via hydrogen bonds, ionic bonds, etc. As nanoscale rigid rod crystals, attapulgite acts like “reinforcing bars” in a polymer matrix, effectively transferring and dispersing stress, significantly enhancing the composite’s strength, modulus, and toughness.[16] Attapulgite is rich in essential trace elements like magnesium and silicon. Studies show magnesium ions can promote chondrocyte metabolism and type II collagen synthesis, while soluble silicic acid has been proven to stimulate chondrocyte proliferation and promote collagen and proteoglycan secretion.[17]

Polyvinyl alcohol (PVA) is a water-soluble synthetic polymer with excellent film-forming ability, chemical stability, and biocompatibility. However, it lacks cell recognition sites, and pure PVA hydrogels are often too dense, hindering cell migration and nutrient diffusion.[18] Combining attapulgite with PVA creates a significant synergistic effect. Attapulgite nanorods form a robust nanocomposite network with PVA molecular chains through physical entanglement and multiple hydrogen bonding interactions. This can increase the compressive modulus and fracture energy of the composite hydrogel by several times or even an order of magnitude compared to pure PVA hydrogel, better simulating and withstanding the complex mechanical environment experienced by articular cartilage.[14] The incorporation of attapulgite significantly alters the rheological behavior of the composite ink. It typically imparts more pronounced shear-thinning properties. Viscosity drops sharply under printing shear force facilitating extrusion and recovers instantly post-extrusion to maintain structural stability. Simultaneously, the nanorod network can increase the ink's yield stress, preventing collapse of lower layers during multi-layer printing, significantly improving shape fidelity.

The surface of the composite hydrogel becomes rougher and richer in active groups due to attapulgite introduction, greatly improving the adhesion, spreading, and proliferation of cells (eg, chondrocytes, MSCs/Mesenchymal Stem Cells/ Mesenchymal Stromal Cells). Magnesium and silicon ions released from attapulgite can create a pro-chondrogenic microenvironment.[17] The high specific surface area and adsorption capacity of attapulgite make it an ideal carrier for growth factors (eg, TGF-β3, BMP-2). Factor-loaded composite hydrogels enable long-term, controlled release, avoiding burst release failure.[19] Preliminary studies suggest some silicate materials can influence the immune microenvironment by regulating macrophage behavior. Attapulgite may possess similar potential, offering a new material basis for constructing "immunomodulatory" cartilage repair scaffolds.

Using attapulgite-PVA composite hydrogel as a bioink has shown potential in constructing high-performance cartilage organoids. Studies indicate that 3D printing of this composite ink loaded with bone marrow mesenchymal stem cells, under chondrogenic induction, can significantly promote cartilage-specific gene expression and glycosaminoglycan deposition, and the formed tissue-engineered cartilage exhibits mechanical properties close to native cartilage.[20] Natural hydrogels are bioactive but mechanically weak (<50 kPa vs. native cartilage 0.5–10 MPa); synthetic polymers (eg, PEG, PVA) offer tunable mechanics but are bio-inert; composites (eg, attapulgite-PVA) provide enhanced mechanics yet face dispersion challenges; and Decellularized Extracellular Matrix (dECM) is highly biomimetic but suffers from batch variability. Therefore, selection should be application-driven: dECM for in vitro models, composites for load-bearing repair, and synthetics for regulatory compliance. Table 3

Smart and Dynamic Responsive Bioinks

The new generation of bioinks aims to simulate the dynamic, reconfigurable nature of native ECM. Supramolecular hydrogels self-assemble based on non-covalent forces like host-guest interactions, hydrogen bonds, and ionic

Table 3 Summary of the Properties of Attapulgite-Polyvinyl Alcohol Composite Hydrogel

Property Dimension	Specific Performance and Mechanism	Contribution to Cartilage Printing
Mechanical Properties	Attapulgite serves as a nano-reinforcing phase, bonding with PVA through hydrogen bonding and entanglement, significantly enhancing compressive strength, modulus, and toughness.	Enables the printed structure to simulate and withstand the mechanical load of articular cartilage, preventing post-implantation collapse.
Rheological & Printability Properties	Introduces significant shear-thinning behavior and higher yield stress, improving shape fidelity and structural resolution.	Facilitates high-precision and high-fidelity printing of complex cartilage structures (e.g., auricle, meniscus).
Bioactivity	Provides cell adhesion sites; slowly releases cartilage-promoting ions such as magnesium and silicon; efficiently loads and sustains the release of growth factors.	Actively guides chondrogenic differentiation of cells and promotes the synthesis and deposition of functional extracellular matrix.
Structural Biomimicry	Nano-rod crystals and polymer networks form a multi-level, anisotropic microstructure that simulates the nanofiber network of natural cartilage ECM.	Provides cells with a physical microenvironment closer to nature, facilitating cell polarization and tissue maturation.

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coordination. They possess excellent shear-thinning and self-healing properties, extruding smoothly during printing and rapidly recovering structure afterward, protecting cells from prolonged shear stress.[21] Stimulus-responsive hydrogels can respond to external signals (eg, temperature, pH, light, enzymes). For example, thermo-sensitive materials (eg, poly (N-isopropylacrylamide) and its copolymers) are printable sols at low temperatures and automatically transition to gels at physiological temperature, simplifying the printing process;[22] enzyme-sensitive hydrogels allow cells to actively remodel the surrounding matrix by secreting specific enzymes (eg, MMPs/Matrix Metalloproteinases), promoting cell migration and tissue integration.[23] Figure 4

Extracellular Matrix-Mimicking Bioinks

In the development of ECM-mimicking biomaterials, the widely used Matrigel, while providing basic support for organoid culture, has driven the development of better-performing biomimetic materials due to its inherent batch-to-batch variability and complexity. Hydrogels, with their tunable mechanical and biochemical properties, have become an ideal ECM-mimicking platform and have further given rise to ECM-mimicking inks, ie, bioinks for 3D bioprinting.[24] By integrating defined ECM components (eg, gelatin, hyaluronic acid, collagen, or synthetic polymers), these inks enable precise design of the microenvironment. For instance, GelMA (gelatin methacryloyl) ink has been successfully used to print tissue constructs with nascent vascular networks;[25] similarly, alginate-based hydrogel inks modified with RGD peptides can support the formation and functional maintenance of liver organoids;[26] furthermore, recently developed thermo-sensitive PLGA-PEG-PLGA copolymer inks can self-assemble into biomimetic scaffolds post-printing for cartilage repair.[27] Additionally, multi-material printing systems allow combining different inks within a single construct to simulate heterogeneous tissue interfaces (eg, osteochondral junction). In the future, such inks will not only drive the fabrication of complex organoid models and personalized tissue implants but can also be integrated with microfluidic

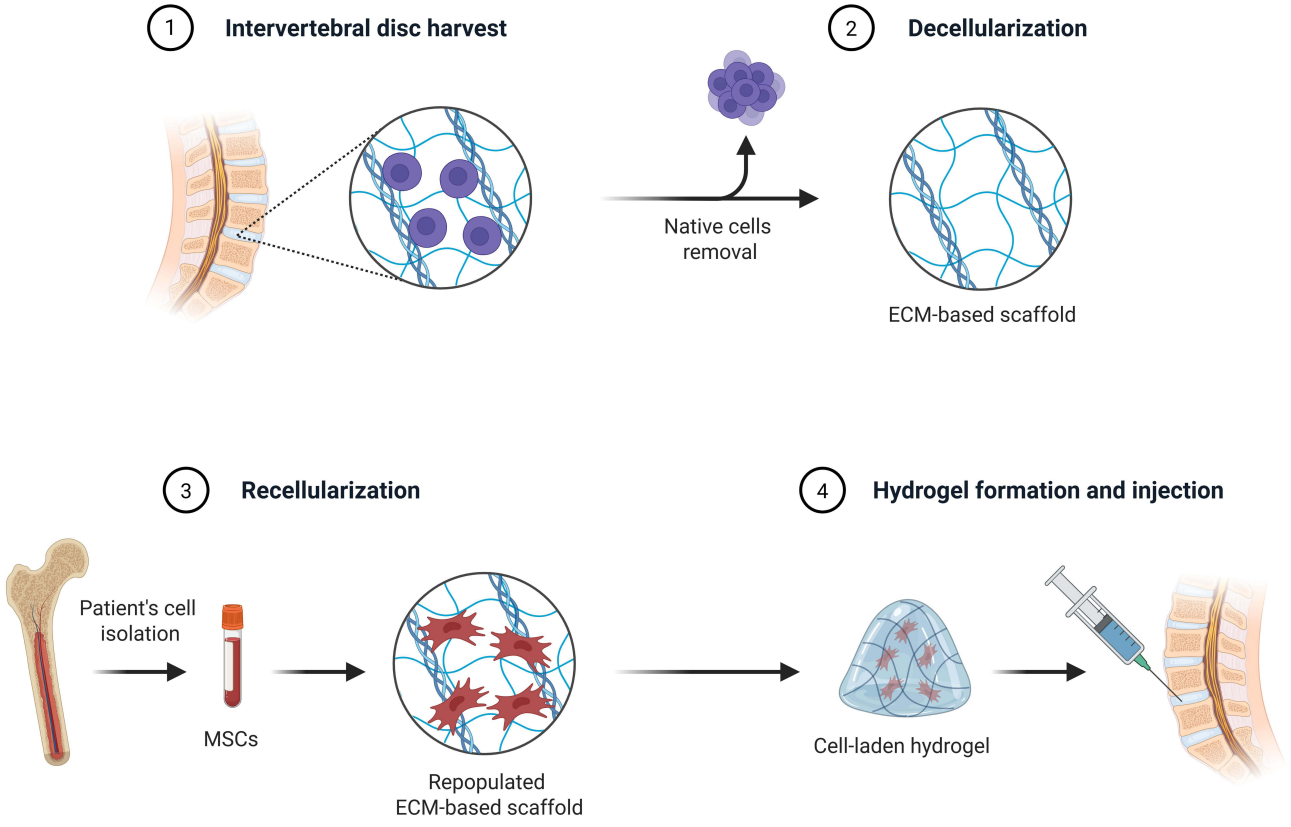

Figure 4 This schematic illustrates an extracellular matrix (ECM)–based strategy for intervertebral disc regeneration. Intervertebral disc tissue is first harvested and subjected to decellularization to remove native cells while preserving the ECM architecture, resulting in an ECM-based scaffold. Patient-derived cells, such as mesenchymal stem cells (MSCs), are then isolated and used to recellularize the scaffold. The recellularized ECM is subsequently processed into a cell-laden hydrogel and injected into the degenerated intervertebral disc, providing a biomimetic microenvironment to support cell survival, matrix remodeling, and disc regeneration.

chips to build "organ-on-a-chip" platforms for drug screening. Through continuous integration of biomimetic design, smart responsive materials, and precision manufacturing technologies, ECM-mimicking inks are gradually achieving more realistic and controllable reproduction of the human microenvironment, providing powerful tools for regenerative medicine and translational research.[28]

Three-Dimensional Bioprinting Technologies

3D bioprinting technology is a key tool for realizing the spatial structure of complex cartilage organoids. Different main technological pathways, based on distinct forming principles, have their own characteristics. The choice depends on a comprehensive consideration of target structure complexity, printing resolution, cell viability requirements, and material properties.[29] Table 4 and Figure 5

Extrusion-Based Bioprinting

As the most prevalent technology, extrusion-based printing uses pneumatic or mechanical (piston/screw) drives to continuously extrude bioink into filaments for layer-by-layer deposition. Its advantage lies in good compatibility with high-viscosity, high-cell-density bioinks, making it suitable for constructing large-volume tissues. Challenges include potential cell damage from high shear stress during extrusion and relatively low resolution (typically >100 μm).[30] Optimization strategies include: developing bioinks with excellent shear-thinning properties to reduce extrusion resistance; using coaxial extrusion printing to create hollow fibers simulating blood vessels or chondrocyte lacunae; employing suspension printing techniques, where bioink is printed into a support bath composed of microgels (eg, gelatin microparticles), enabling the fabrication of complex, unsupported overhanging structures.[31,32]

Light-Curing Bioprinting

This technology utilizes principles like Digital Light Processing (DLP) or Stereolithography (SLA) to selectively irradiate photosensitive bioink with a specific wavelength light source, causing photo-crosslinking and solidification. Its core advantage is extremely high printing resolution (up to 10–50 μm or even sub-micron level), enabling precise replication of cartilage tissue's microscopic structures, such as fine pores and surface topography. DLP technology, due to

Table 4 Comparison of Major Three-Dimensional Bioprinting Technologies

Printing Technology	Forming Principle	Resolution	Advantages	Disadvantages	Focus in Cartilage Applications
Extrusion-based	Mechanical/ Pneumatic Continuous Extrusion	100–500 μm	Broad material compatibility, can print high cell density, moderate cost	Shear stress may damage cells, relatively low resolution	Large-sized cartilage/ osteochondral composite scaffolds, organoid precursors
Photopolymerization	Photo-induced Layer/Surface Curing	10–100 μm	Extremely high resolution, good surface finish, fast printing speed (especially DLP)	Potential toxicity of photoinitiators/UV light, limited to photosensitive materials	High-precision microstructures (e.g., biomimetic lacunae), detailed cartilage models
Inkjet	Thermal Bubble/ Piezoelectric Droplet Generation	50–300 μm	High speed, low cost, suitable for patterning	Limited ink viscosity/cell density, droplet impact force, weak structural strength	Patterning deposition of cells/growth factors, high-throughput drug screening models
Microfluidic Bioprinting	Precise Fluid Manipulation within Microchannels	Variable	Can create continuous gradients, online material mixing, microenvironment simulation	Complex system, high design and fabrication difficulty	Gradient interface structures (e.g., bone-cartilage junctions), vascular network prototypes

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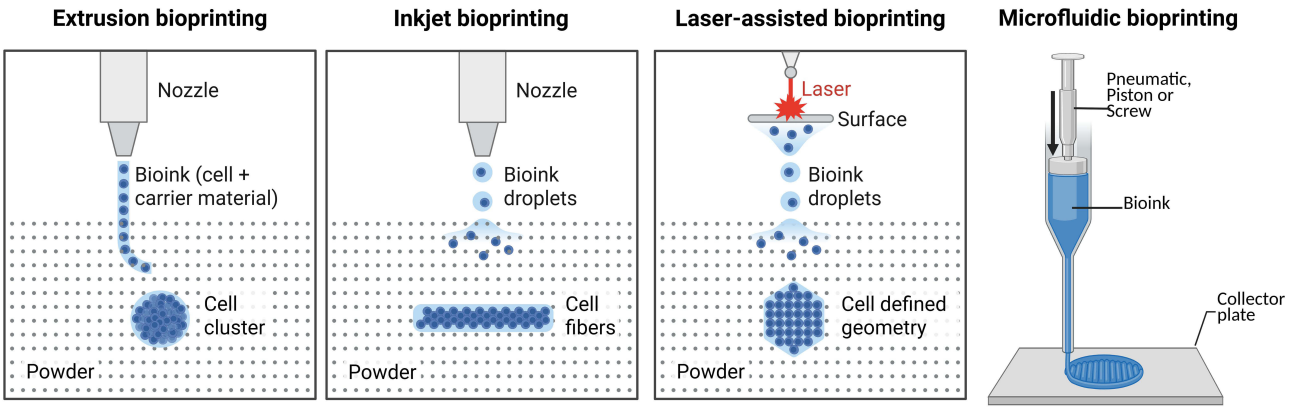

Figure 5 This figure illustrates the main types of additive manufacturing techniques used in bioprinting processes. Extrusion-based bioprinting deposits continuous filaments of cell-laden bioinks through a nozzle to generate three-dimensional cell clusters. Inkjet bioprinting dispenses bioink droplets in a drop-on-demand manner, enabling the formation of cell fibers and patterned constructs. Laser-assisted bioprinting uses laser-induced forward transfer to precisely deposit bioink droplets with defined cellular geometry. Microfluidic bioprinting employs controlled fluid flow, driven by pneumatic, piston, or screw mechanisms, to generate structured bioink constructs collected on a substrate. Together, these approaches provide complementary capabilities for fabricating complex, cell-laden architectures in tissue engineering and regenerative medicine.

its layer projection curing characteristic, offers significantly faster printing speeds than SLA's point scanning while maintaining high accuracy. Main challenges are the potential cytotoxicity of photoinitiators and possible DNA damage from UV light to cells. Solutions include developing visible light initiation systems and photoinitiators with better biocompatibility.[33–35]

Inkjet Bioprinting

Inkjet printing generates droplets via thermal bubble or piezoelectric effect to eject bioink on demand. Its characteristics are fast printing speed, relatively high resolution, and lower cost, making it suitable for printing cell patterns and high-throughput screening arrays. Limitations are evident: it typically can only use low-viscosity, low-cell-concentration inks to avoid nozzle clogging; droplet impact may affect cell viability; it is difficult to construct high-strength 3D structures. Therefore, it is often combined with other technologies or used for patterned deposition of bioactive factors and specific cells.[34,36]

Emerging and Hybrid Printing Strategies

Microfluidic bioprinting achieves online mixing and real-time regulation of multiple cells or materials during printing by integrating microfluidic chips into the printhead, enabling precise generation of composition or cell density gradients. This is crucial for constructing biomimetic gradient interface structures like the osteochondral transition layer.[37,38] Acoustic printing utilizes acoustic wave energy for nozzle-free, non-contact manipulation and assembly of cells and microcarriers, fundamentally avoiding shear stress damage to cells, significantly improving cell survival rates, and being particularly suitable for high-precision spatial arrangement of shear-sensitive cells.[39] Multimodal or hybrid printing strategies synergistically combine the advantages of multiple printing technologies. For example, first printing a synthetic polymer (eg, PCL) framework via fused deposition modeling (FDM) to provide mechanical support, and then filling its pores with cell-laden hydrogels via extrusion-based printing. This cleverly balances the macro-structural mechanical strength with the micro-environmental bioactivity within a single construct.[40] These emerging and hybrid printing strategies jointly promote the development of cartilage organoid manufacturing towards higher biomimicry, better cell compatibility, and stronger structural-functional integration.[41]

Functionalization Strategies

Constructing morphologically realistic cartilage organoids is only the first step. How to mature them in vitro and achieve long-term stable function after implantation in vivo is a more challenging task.[42]

Cell Sources and Co-Culture Systems for Cartilage Organoids

Cell Sources

The construction of cartilage organoids (CORGs) begins with the selection and directed differentiation of suitable cell sources.[34] As natural cartilage tissue is primarily composed of a single cell type—chondrocytes—CORG culture mainly follows two pathways: one involves directly obtaining and culturing primary chondrocytes isolated from cartilage tissues like joints, nose, or ear;[43] the other involves in vitro differentiation of pluripotent stem cells into chondrocytes before culture. Direct use of primary chondrocytes, especially autologous articular chondrocytes, is considered the "gold standard" and maximizes functional phenotype retention. However, its source is extremely limited, prone to dedifferentiation (loss of specific function) during in vitro expansion, and suffers from donor variability and declining proliferative capacity with donor age.[44] Therefore, stem cells with self-renewal and multipotent differentiation capabilities have become more attractive alternatives. Among them, mesenchymal stem cells (MSCs), particularly easily accessible bone marrow-derived (BMSCs) and adipose-derived (ADSCs) MSCs, have become a research hotspot due to their well-defined chondrogenic induction capacity and relatively low ethical controversy. They have been successfully used in multiple studies to construct CORGs and repair cartilage defects in animal models.[45] Induced pluripotent stem cells (iPSCs), obtained by reprogramming somatic cells, provide an unlimited, patient-specific source of chondrocytes for individualized therapy and have shown repair potential in primate models. However, their differentiation purity, genetic stability, and potential tumorigenic risk require further optimization and evaluation.[42] Additionally, embryonic stem cells (ESCs), while possessing the strongest pluripotency, face severe ethical challenges, and related research remains in early stages.[46] Overall, using stem cell differentiation to obtain chondrocytes is currently the most promising strategy. Future research needs not only to develop more stable, safe, and efficient differentiation protocols but also to address key issues like the functional maturity of stem cell-derived chondrocytes, long-term in vivo phenotypic stability, and standardization of scalable production to effectively promote CORG translation from basic research to clinical therapy.[3,47]

Certainly, beyond the primary chondrocytes, MSCs, iPSCs, and ESCs detailed above, there are other potential cell sources and directed differentiation strategies for constructing cartilage organoids, providing complementary approaches to overcome existing challenges.[48]

Direct reprogramming (transdifferentiation) of fibroblasts is a technique that uses specific transcription factors or small molecule compounds to directly reprogram somatic cells (eg, skin fibroblasts) into induced chondrocytes or chondroprogenitor cells without passing through a pluripotent stem cell state.[49] This method bypasses the tumorigenic risk associated with iPSCs and may yield a phenotype closer to mature chondrocytes, offering another faster and theoretically safer route to obtain patient-specific cells.[50] The synovium is the inner lining of the joint capsule. Synovium-derived MSCs or synovial cells themselves have been shown to possess certain chondrogenic differentiation potential. Utilizing cells derived from the joint microenvironment itself may be more conducive to constructing cartilage organoids biologically compatible with host tissue and promoting integration during repair.[51] Small populations of proliferative and differentiable progenitor cells exist in mature cartilage tissue (especially in the superficial zone or cartilage-bone interface).[52] Although isolation and purification are difficult, these cells are inherently directed towards the chondrogenic lineage, potentially offering more stable and efficient chondrogenic capacity than pluripotent stem cells, making them a highly promising autologous cell source.[53] Other cells like periosteal cells, dental pulp stem cells, etc, have also been confirmed to have chondrogenic differentiation potential, providing alternative options for cartilage repair in specific contexts.[54]

In terms of directed differentiation strategies, beyond traditional growth factor (eg, TGF-β superfamily) induction, emerging methods are continually emerging. Epigenetic regulation using small molecule drugs to modulate chromatin state can more effectively drive and maintain the expression program of chondrocyte-specific genes.[55] Applying specific biophysical stimuli (eg, dynamic compression, shear stress) during differentiation, synergizing with biochemical signals, can guide stem cells towards more functionally mature chondrocyte differentiation.[56] Using decellularized cartilage matrix (dECM) hydrogels or functionalized synthetic materials to provide cells undergoing differentiation with physical and biochemical cues closest to the native state has been proven to significantly improve differentiation efficiency and cell function.[57]

In summary, the selection of cell sources for cartilage organoids is becoming increasingly diverse, and directed differentiation strategies are also evolving from single biochemical factor induction to a multidimensional regulatory system integrating genetics, epigenetics, mechanobiology, and biomimetic materials science.[58] Future research will focus on screening and optimizing these alternative cell sources and integrating multimodal differentiation strategies to obtain functionally mature, phenotypically stable chondrogenic building units suitable for scaled clinical application.[59]

Co-Culture Strategies

After identifying suitable cell sources for cartilage organoids (CORGs), creating a three-dimensional growth environment that can simulate the structure and function of native cartilage tissue is crucial. This environment should not only integrate key extracellular matrix (ECM) components to promote vital cell-matrix interactions but also provide greater surface area and permeability, ensuring efficient nutrient and oxygen access for chondrocytes and supporting tissue growth.[60]

Currently, in vitro culture of CORGs mainly follows two models: scaffold-free and scaffold-based.[61] Scaffold-free culture relies on the self-assembly capability of cells, promoting spontaneous aggregation of chondrocytes into clusters (eg, microspheres) in suspension systems. While this method avoids the influence of foreign materials, facilitates cell purification, and allows cells to form structures with native-like characteristics through autonomous secretion and reorganization of ECM, it faces significant challenges in precisely controlling organoid size, structure, and maintaining long-term mechanical function, requiring further research and optimization.[62,63]

Given the central role of ECM in providing mechanical support, biological signals, and structural templates, scaffold-based culture has become the mainstream strategy for constructing CORGs.[34] This method utilizes biodegradable biomimetic materials as a three-dimensional framework, aiming to reconstruct an ECM-like microenvironment supporting cell growth, proliferation, and differentiation. Common scaffold materials include natural/decellularized matrices, various hydrogels, and biosynthetic polymers.[64] By finely tuning scaffold material composition (eg, modifying hyaluronic acid with dopamine to enhance bioactivity), structural parameters (eg, designing microchannels to improve nutrient delivery and cell communication), and mechanical properties (eg, adjusting stiffness to optimally promote MSC migration and chondrogenic differentiation), the culture environment can be greatly optimized to guide the formation of functional cartilage tissue.[65]

Furthermore, to simulate complex inter-tissue interactions in vivo and enhance repair outcomes, advanced culture strategies introduce co-culture systems.[66] For example, co-culture with subchondral bone cells (eg, osteoblasts or MSCs) can simulate the physiological dialogue within the osteochondral unit, promoting the formation of a functional interface;[67] while co-culture with endothelial cells, although not native components of cartilage, can construct a prevascularized network at the organoid periphery or base. This is crucial for repairing large defects, aiding rapid establishment of connection with host blood circulation post-implantation, thereby ensuring nutrient supply and long-term survival in the core region of the organoid.[68] In summary, comprehensively applying and optimizing scaffold-based and co-culture strategies is a key path to constructing structurally biomimetic, functionally mature cartilage organoids with in vivo integration potential. Current in vitro strategies for cartilage organoid culture can be broadly divided into scaffold-free and scaffold-based models. Scaffold-free systems rely on cellular self-organization to generate spheroids or microtissues, thereby avoiding the use of exogenous supporting materials. Although this approach is beneficial for preserving cell-driven assembly, it is limited by poor control over construct size, morphology, and long-term mechanical properties. Scaffold-based systems, including porous scaffolds and 3D-bioprinted constructs, overcome some of these limitations by providing predefined structural guidance. In particular, 3D bioprinting offers distinct advantages in producing patient-specific, anatomically compatible constructs with spatially defined distributions of cells and biomaterials, making it especially suitable for mimicking the structural complexity of native cartilage.Figure 1

Direct Vascularization Potential of Cartilage Organoids

Achieving effective vascularization of cartilage organoids is crucial for their survival, maturation, and repair of large-scale defects.[69] Since the diffusion distance of oxygen and nutrients in tissue typically does not exceed 200 micrometers, traditional organoids relying solely on passive diffusion are highly prone to necrosis in the core region due to nutrient

deprivation and hypoxia when scaled up.[70] Therefore, constructing an internal vascular network is key to breaking the size limitation of organoids. Current vascularization strategies mainly include the following approaches: co-culture with angiogenesis cells, integration with pre-fabricated vascular organoids, utilization of the in vivo environment for in vivo vascularization, and direct construction using bioprinting.[71]

Co-culture with angiogenesis cells is the most commonly used strategy, typically co-seeding chondrocytes or MSCs with human umbilical vein endothelial cells, endothelial progenitor cells, etc.[66] However, a single endothelial cell type struggles to form mature vascular structures; thus, more advanced schemes tend to use multicellular co-culture systems including endothelial cells, fibroblasts, and pericytes to simulate a more complex microvascular environment.[72] Vascular organoid integration involves co-assembling cartilage organoids with pre-cultured vascular organoids in the early stage of construction, aiming for natural infiltration and connection of endogenous vascular networks. However, the compatibility of different organoid culture conditions is a major challenge for this method.[73] In vivo vascularization strategy involves implanting in vitro constructed cartilage organoids into a host (eg, subcutaneously or into the joint cavity), leveraging the host's strong angiogenic capacity to allow host vessels to grow into the organoid.[42] Using organoids constructed from autologous cells for such transplantation minimizes immune rejection risk and has significant clinical translation potential.

However, these methods relying on cell self-assembly or host ingrowth often suffer from issues like disorganized vascular network structure, long formation cycles, and low perfusion efficiency.[74] In recent years, 3D bioprinting technology has provided revolutionary tools for directly manufacturing precisely controllable vascular networks. Among them, the sacrificial ink method is particularly prominent: using soluble materials like gelatin, Pluronic F127, or PVA as sacrificial templates, they are co-deposited with cell-laden bioinks (eg, GelMA) via multi-nozzle or coaxial printing technology. Subsequently, removing the sacrificial material by dissolution or melting leaves behind a complete, interconnected, and perfusable microchannel network within the organoid.[75] This method not only significantly improves internal mass transport, promoting long-term cell viability and function, but also provides pre-designed physical channels for subsequent endothelialization, offering a powerful technical framework for constructing large-scale, vascularized cartilage organoids that are structurally biomimetic and functionally sound.[76,77]

Cellular Microenvironment in Cartilage Repair

As a key component of the cartilage repair microenvironment, chondrocytes play a pivotal role in the regeneration process. They synthesize and secrete extracellular matrix (ECM) components such as type II collagen and proteoglycans, which are crucial for maintaining cartilage structure and function.[78] Allogeneic chondrocyte implantation into cartilage defects can promote ECM production, improve the biomechanical properties of regenerated cartilage through integration with host tissue, and thus facilitate the repair process.[79] Additionally, chondrocytes release anti-inflammatory cytokines and growth factors, supporting tissue repair and modulating the local microenvironment, further promoting cartilage regeneration and healing.[80] However, it is important to note that chondrocytes inherently possess limited intrinsic repair capacity. Nonetheless, under certain conditions, inducing these cells to secrete repair factors or actively participate in the repair process may be beneficial, thereby enhancing their contribution to cartilage regeneration.[81] This cellular activity underscores the importance of chondrocytes in formulating effective cartilage repair therapeutic strategies.

Spatiotemporal Delivery of Bioactive Factors

Cell-scaffold composites alone are often insufficient to drive adequate cartilage formation. Integration of growth factors (eg, TGF-β superfamily members, BMPs, IGF-1) is needed to guide cell differentiation and matrix synthesis. Smart delivery systems are key, such as encapsulating growth factors in gelatin microspheres, PLGA nanoparticles, or conjugating them to heparinized hydrogels to achieve slow, controlled release, or even release in response to specific cellular activities (eg, enzymatic degradation), mimicking spatiotemporal signals during development.[82,83]

Cytokine and Chemokine Regulatory Network

In the bioprinting of cartilage organoids, scaffolds provide physical support and microenvironment, cells serve as building blocks, while cytokines and chemokines are the "biological instructions" and "chemical navigation" guiding cell behavior and coordinating tissue development and repair. They constitute a complex spatiotemporal signaling

network precisely regulating cell survival, proliferation, migration, differentiation, and matrix metabolism. Effectively integrating this regulatory network into the 3D bioprinting system is the core and difficulty in achieving functional cartilage regeneration.[84,85] Table 5

Core Cytokine Network and Integration Strategies in Printing

The regulation of key cytokine networks, such as growth factors, plays a crucial role in cartilage homeostasis and regeneration.[86] Among them, TGF-β1 and TGF-β3 promote the expression of SOX9 by activating the SMAD2/3 pathway, thereby enhancing the synthesis of type II collagen and aggrecan. Their sustained and appropriate signaling is critical for preventing post-printing cell dedifferentiation or fibrosis.[87] BMP-2, BMP-4, and BMP-7 synergize with TGF-β in early chondrogenesis, but improper control of signal intensity and timing can lead to chondrocyte hypertrophy and terminal differentiation.[88,89] IGF-1 promotes anabolic metabolism, has anti-apoptotic effects, and particularly improves the survival rate of cells in the core region of large-scale organoids.[90] FGF-2 is commonly used to expand MSCs prior to printing, while FGF-18 shows potential in promoting cartilage formation and preventing cartilage degradation.[91] However, directly mixing cytokines into bioinks often leads to burst release and rapid loss of efficacy. Therefore, spatiotemporally controlled delivery strategies are needed: for example, encapsulating TGF-β3 and IGF-1 in degradable polymer microparticles to achieve sustained release over several weeks to months;[92] or incorporating heparin or heparin-binding domain peptides into the hydrogel network to protect growth factors from degradation through high-affinity binding;[93] another method involves printing genetically engineered MSCs that secrete specific cytokines (though this requires strict safety evaluations). On the other hand, chemokines primarily guide the migration of cells: the SDF-1α/CXCR4 signaling axis is an important stem cell homing pathway, and loading SDF-1α into printed scaffolds can actively recruit host endogenous MSCs.[94,95] CCL5, CCL25, and others also exert chemotactic effects on specific MSC subpopulations.[96] In the inflammatory response accompanying cartilage injury, pro-inflammatory chemokines like MCP-1/CCL2 and IL-8 recruit monocytes/macrophages and neutrophils,[97,98] while anti-inflammatory/repair chemokines like MDC/CCL22 are associated with the recruitment of M2-type reparative macrophages.[99] Therefore, designing

Table 5 Functions and Delivery Strategies of Key Cytokines/Chemokines in Cartilage Organoid Bioprinting

Factor Category	Key Representatives	Primary Biological Functions	Challenges in Bioprinting Integration	Advanced Delivery Strategies
Chondrogenic Factors	TGF-β1, TGF-β3	Induce chondrogenic differentiation of MSCs, maintain chondrocyte phenotype, promote synthesis of type II collagen and proteoglycans	Require sustained, stable, and dosage-controlled release; improper dosage or timing may lead to fibrosis or hypertrophy	Heparin-functionalized hydrogels for binding and sustained release; genetically engineered cells for autocrine delivery
Anabolic/ Survival Factors	IGF-1	Promote cartilage matrix synthesis, inhibit cell apoptosis, mediate mechanotransduction signaling	Need to ensure survival of cells in the core region of large-scale constructs	Co-delivery with TGF-β for synergistic, sustained release; smart release systems responsive to mechanical loading
Chemokines	SDF-1α (CXCL12)	Potently recruit CXCR4-expressing MSCs to the injury site for homing	Require simulation of physiological concentration gradients; short in vivo half-life	Creating concentration gradients within scaffolds; covalent immobilization to scaffold materials to prolong activity
Inflammation-modulating Factors	IL-4, IL-10 (Anti-inflammatory); Anti-CCL2 strategies (Anti-catabolic)	Polarize macrophages towards the M2 reparative phenotype; suppress excessive inflammatory responses	Require precise spatiotemporal control to match the inflammatory phase and avoid immunosuppressive risks	"Smart" release systems responsive to the inflammatory microenvironment (e.g., pH, ROS)

immunomodulatory bioinks can involve controllably releasing anti-inflammatory cytokines (eg, IL-4, IL-10) to induce M2 macrophage polarization, while integrating chemokine receptor antagonists or neutralizing antibodies to suppress excessive pro-inflammatory signals, thus fostering a regenerative immune microenvironment.

Signal Synergy and Advanced Delivery Technologies

Successful regeneration relies on the orderly synergy of multiple signals. For example, early repair may require SDF-1α to recruit stem cells, followed by TGF-β3 dominating chondrogenic differentiation, with IGF-1 supporting matrix synthesis, and later inhibition of excessive BMP signaling to prevent hypertrophy.[100] Multi-stage release systems: Integrating multiple carriers with different release kinetics within the same scaffold. For instance, loading SDF-1α onto fast-degrading microparticles for early release to recruit cells, and loading TGF-β3 onto slow-degrading microparticles for long-term release to guide differentiation.[101] Stimuli-responsive smart materials: Developing materials that release encapsulated repair factors in response to specific microenvironmental changes (eg, acidic pH at injury sites, overexpressed MMPs). This enables "on-demand release," improving treatment targeting and safety. Gene-activated matrices: Combining plasmid DNA or mRNA encoding specific cytokines or chemokines with biomaterials. After cellular uptake, the cells themselves become "mini bioreactors," continuously producing the required therapeutic protein.[102] This offers the possibility of ultra-long-term signal regulation.

Integrating the precise regulatory network of cytokines and chemokines into the 3D bioprinting system marks an important leap in this field from "passive support" to "active guidance" of regeneration. The future direction is to develop smarter, more biomimetic multi-signal delivery platforms capable of dynamically responding to in vivo microenvironmental changes, ultimately achieving cartilage regeneration that is fully biomimetic in structure, function, and biological signals.

Dynamic Mechanical Stimulation and Bioreactor Culture

Chondrocyte phenotype and metabolism are highly dependent on their mechanical environment. Static culture fails to replicate the cyclic compression, shear, and fluid stresses articular cartilage endures in vivo.[103] Bioreactor application is crucial. Perfusion bioreactors provide nutrients and shear stress via medium flow; compression bioreactors simulate cyclic joint loading; combined loading systems can provide multimodal mechanical stimulation closer to physiology.[104] These dynamic culture conditions have been confirmed to significantly enhance ECM deposition in cartilage organoids, improve their mechanical properties, and inhibit hypertrophic differentiation leading to calcification.

Immunomodulation and Pro-Integration Strategies

Foreign body reaction and chronic inflammation triggered by implants are important causes of repair failure. New-generation cartilage organoid designs are beginning to incorporate immunomodulatory functions. For example, using bioinks containing interleukin-1 receptor antagonist (IL-1Ra) or macrophage polarization regulators (eg, IL-4) can actively polarize infiltrating macrophages from a pro-inflammatory (M1) phenotype to an anti-inflammatory/pro-repair (M2) phenotype, fostering a microenvironment conducive to regeneration and promoting graft integration with host tissue.[105,106] Table 6, Figure 6

Osteoimmunomodulation: Key Roles of Macrophages, Neutrophils, and NETs in Cartilage Repair and Bioprinting

In recent years, osteoimmunology has reshaped our understanding of skeletal physiology and pathology by highlighting the dynamic interactions between immune cells and bone/cartilage cells. During cartilage injury and repair, innate immune cells—especially macrophages and neutrophils—play a double-edged role. They can help resolve inflammation and support regeneration, but they may also drive chronic inflammation and matrix breakdown. Therefore, incorporating osteoimmunomodulatory strategies into 3D bioprinted cartilage organoids has become a promising way to improve in vivo survival, integration, and long-term stability.[107]

Macrophages are among the first immune cells to arrive at injured cartilage, and their polarization strongly affects repair outcomes. M1 macrophages, induced by IFN-γ, LPS, or damage-associated signals, release pro-inflammatory cytokines, reactive oxygen species, and matrix metalloproteinases (MMPs) to remove debris and pathogens.[108] However, prolonged M1

Table 6 Role of Key Osteoimmune Cells/Components in Cartilage Repair and Their Bioprinting Regulation Strategies

Immunological Component	Major Subtype/ State	Role in Cartilage Injury Repair	Potential Harm to Bioprinted Organoids	Strategies for Bioprinting Integration Control
Macrophage	M1 type	Clears debris and initiates inflammation; excessive activity leads to persistent inflammation and matrix degradation.	May cause inflammatory erosion around the graft, inhibit cellular function, and lead to implant degradation.	Material-induced polarization (topography, stiffness, IL-4 immobilization); active delivery of regulators (Resolvin D1, anti-IL-1β); co-printing with M2 cells.
Macrophage	M2 type	Secretes anti-inflammatory and repair factors, promotes angiogenesis, matrix remodeling, and tissue repair.	Beneficial, but requires induction within the appropriate time window.	Similar to above, aiming to promote M2 polarization.
Neutrophil	Activated/ NETosis	Provides early defense; excessive activation leads to NET formation, causing tissue damage and chronic inflammation.	NETs can directly attack implanted cells, trigger autoimmune responses, and hinder integration.	Delivery of NET scavengers (DNase I microspheres); delivery of protease inhibitors (NE/MPO inhibitors); material design resistant to NETs.
Neutrophil Extracellular Traps (NETs)	N/A (structure)	Traps pathogens; in pathological states, damages host tissues and sustains inflammation.	Physical and chemical barriers, leading to cell death and integration failure around the implant.	Primarily clearance and inhibition strategies, such as localized sustained release of DNase I.

activation creates a harmful environment that accelerates matrix degradation and suppresses the chondrogenic differentiation of mesenchymal stem cells (MSCs).[109] By contrast, M2 macrophages, stimulated by IL-4, IL-13, and IL-10, produce anti-inflammatory cytokines and growth factors that support ECM remodeling and tissue repair. Successful cartilage regeneration usually depends on a timely shift from early M1 activity to a later M2-dominant phase.[106]

Bioink composition and surface properties can directly influence macrophage behavior. For example, polysaccharide-based hydrogels with micro/nanostructures or immobilized IL-4 have been shown to promote M2 polarization.[110] Bioinks can also be loaded with immunomodulatory molecules, such as IL-4/IL-13 mimetic peptides, Resolvin D1, or nanobodies against IL-1β/TNF-α, to regulate macrophage phenotypes in a spatially and temporally controlled manner.[111] Some studies have further explored co-printing pre-polarized M2 macrophages, or their precursors, together with chondrocytes or MSCs to guide the local immune environment toward repair after implantation.[112]

Neutrophils are the main effector cells in acute inflammation and are rapidly recruited after injury. Recent studies have shown that they contribute to disease progression through the formation of neutrophil extracellular traps (NETs), especially in arthritis.[113] During NETosis, neutrophils release chromatin DNA and granular proteins such as myeloperoxidase (MPO), neutrophil elastase (NE), and histones, forming web-like NET structures.[114] These components can directly damage chondrocytes and endothelial cells. NETs may also carry citrullinated proteins that serve as autoantigens, promoting anti-citrullinated peptide antibody production and contributing to the chronic progression of autoimmune diseases such as rheumatoid arthritis.[115] In addition, their fibrous structure can hinder cell migration and tissue integration, while sustaining inflammation through macrophage and complement activation.[116]

For this reason, incorporating NET-targeting components into biomaterials is an attractive strategy. DNase I, for example, can be loaded into biodegradable microspheres and integrated into bioinks for sustained release, helping clear NETs at the implantation site.[117] Local delivery of MPO or NE inhibitors can further neutralize the toxic protein components of NETs. Surface modification strategies, such as introducing phosphocholine groups or designing specific nanotopographies, may also reduce excessive neutrophil activation and NETosis by improving the anti-inflammatory and anti-biofouling properties of biomaterials.[118]

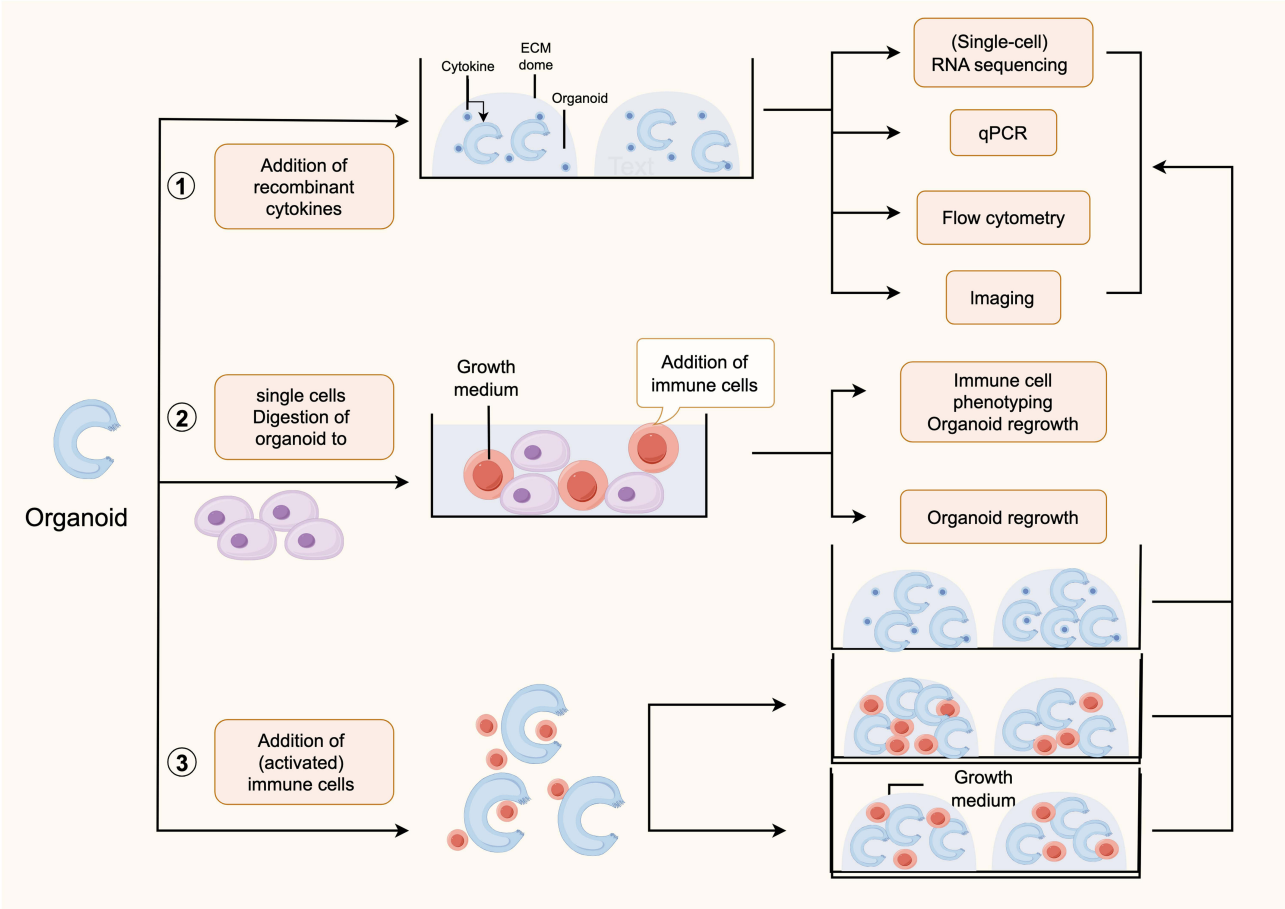

Figure 6 This schematic illustrates experimental strategies for investigating immune regulation and functional responses using organoid models. Recombinant cytokines can be added to organoid cultures to modulate organoid growth and state, followed by molecular and phenotypic analyses including (single-cell) RNA sequencing, qPCR, flow cytometry, and imaging. Organoids can also be dissociated into single cells and co-cultured with immune cells in growth medium to enable immune cell phenotyping and assessment of organoid regrowth. In addition, activated immune cells may be directly introduced into intact organoid cultures to establish organoid–immune co-culture systems, allowing the study of immune cell infiltration, cell–cell interactions, and their effects on organoid growth and remodeling.

Comprehensive Strategy for Constructing "Immunomodulatory" Cartilage Organoids

Future advanced cartilage organoids should move beyond the pursuit of "immune inertness" and instead proactively design and utilize immune responses to promote repair. A comprehensive "immunomodulatory" bioprinting strategy should encompass the following levels. Personalized design: Before printing, analyze the immune microenvironment characteristics of target patients or disease models using technologies like single-cell sequencing to identify key immunomodulatory targets (eg, specific pro-inflammatory factors, chemokines, or NETs-related proteins), enabling precise, personalized design.[119] Phase-responsive materials: Design materials with staged responses. The first stage (0–72 hours post-implantation) rapidly releases NETs inhibitors and M1-to-M2 transition inducers to control acute inflammation.[120] The second stage (days to weeks) continuously releases chondrogenic factors (eg, TGF-β3, IGF-1) and pro-angiogenic factors, driving functional cartilage regeneration within the established favorable immune microenvironment.[121] Pre-implantation conditioning: Before implantation, co-culture the printed cartilage organoid with immune cells like macrophages and neutrophils in a microfluidic chip or bioreactor for a short period. By previewing and adjusting the immune response, the optimal material formulation or surface treatment scheme can be screened before in vivo implantation, improving success rates.

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Current Challenges and Future Perspectives

Challenges and Optimization Strategies in Cartilage Organoid Bioink Development

The development of bioinks for 3D bioprinted cartilage organoids remains a typical multi-objective optimization problem, in which printability, structural fidelity, mechanical performance, cytocompatibility, and biomimicry are closely interconnected and often difficult to satisfy at the same time.[122] To enable precise extrusion and stable shape retention, bioinks generally need shear-thinning behavior together with rapid crosslinking. Yet improving viscosity to enhance printing fidelity usually requires higher extrusion pressure, which can reduce cell viability.[123] A similar trade-off exists between mechanics and bioactivity: naturally derived materials such as gelatin and hyaluronic acid are favorable for cell interaction but often mechanically weak, whereas reinforcement with synthetic polymers or nanomaterials may improve stiffness at the expense of biological function.[124]

Cytocompatibility is further challenged by shear stress during printing, possible toxicity from photoinitiators used in photocuring, and the limited bioactive cues in many base materials.[125] Although decellularized cartilage matrix (dECM) offers a more biomimetic microenvironment, its application is still restricted by source-related risks, batch variability, and ethical concerns. Another major limitation is that current bioinks still struggle to reproduce the zonal heterogeneity of native cartilage, including gradients in matrix composition, cell phenotype, and mechanical properties. This shortcoming becomes even more evident in the fabrication of osteochondral constructs, where multiple cell types must be co-encapsulated while maintaining compatible microenvironments and avoiding adverse crosstalk.[126]

Beyond material design, several broader barriers continue to limit the field. Current technologies are still insufficient to accurately reproduce the nanoscale organization of collagen fibers and proteoglycan distribution found in native cartilage, which constrains the mechanical and tribological performance of engineered tissue. In addition, MSCs and expanded chondrocytes often undergo hypertrophy or fibrocartilaginous drift during long-term culture or after implantation, compromising the stability of the hyaline cartilage phenotype. For larger, clinically relevant constructs, poor mass transport and insufficient vascular support can lead to hypoxia, nutrient deprivation, and cell death in the core region. Clinical translation is also slowed by the lack of standardized protocols and clear regulatory pathways for cell sourcing, bioink preparation, printing procedures, and product evaluation.[126]

Taken together, the central challenge in cartilage organoid bioink development is not the optimization of a single property, but the coordinated improvement of multiple competing requirements. Future progress will likely depend on smart material systems, including dynamic covalent hydrogels and stimuli-responsive bioinks, that can better integrate printability, biological function, and tissue-specific complexity, thereby supporting the fabrication of cartilage constructs with both structural and functional fidelity.[127]

Clinical translation of bioprinted cartilage organoids remains limited by challenges in standardized manufacturing, batch consistency, quality control, long-term safety evaluation, and regulatory classification. In addition, their regulatory pathway is likely to differ across jurisdictions, such as the FDA framework for HCT/Ps and regenerative medicine products in the United States and the ATMP/tissue-engineered product framework in Europe.[128–130]

Future Development Directions

1. "4D" Bioprinting and Smart Materials: Developing smart bioinks that can undergo shape, stiffness, or functional evolution in response to in vivo physiological signals (eg, pH, enzymes, mechanics), enabling post-printing structural adaptation and functional evolution. Figure 7
2. Multi-Technology Integration and Manufacturing Innovation: Combining bioprinting, electrospinning, organ-on-a-chip, and robotic automation to achieve integrated, high-throughput, standardized manufacturing from nanofiber networks to macro-anatomical structures.
3. Organoid-on-a-Chip and Personalized Medicine: Integrating printed cartilage organoids with microfluidic systems to build "joint-on-a-chip" models for patient-specific drug screening and disease mechanism research, enabling true precision medicine.

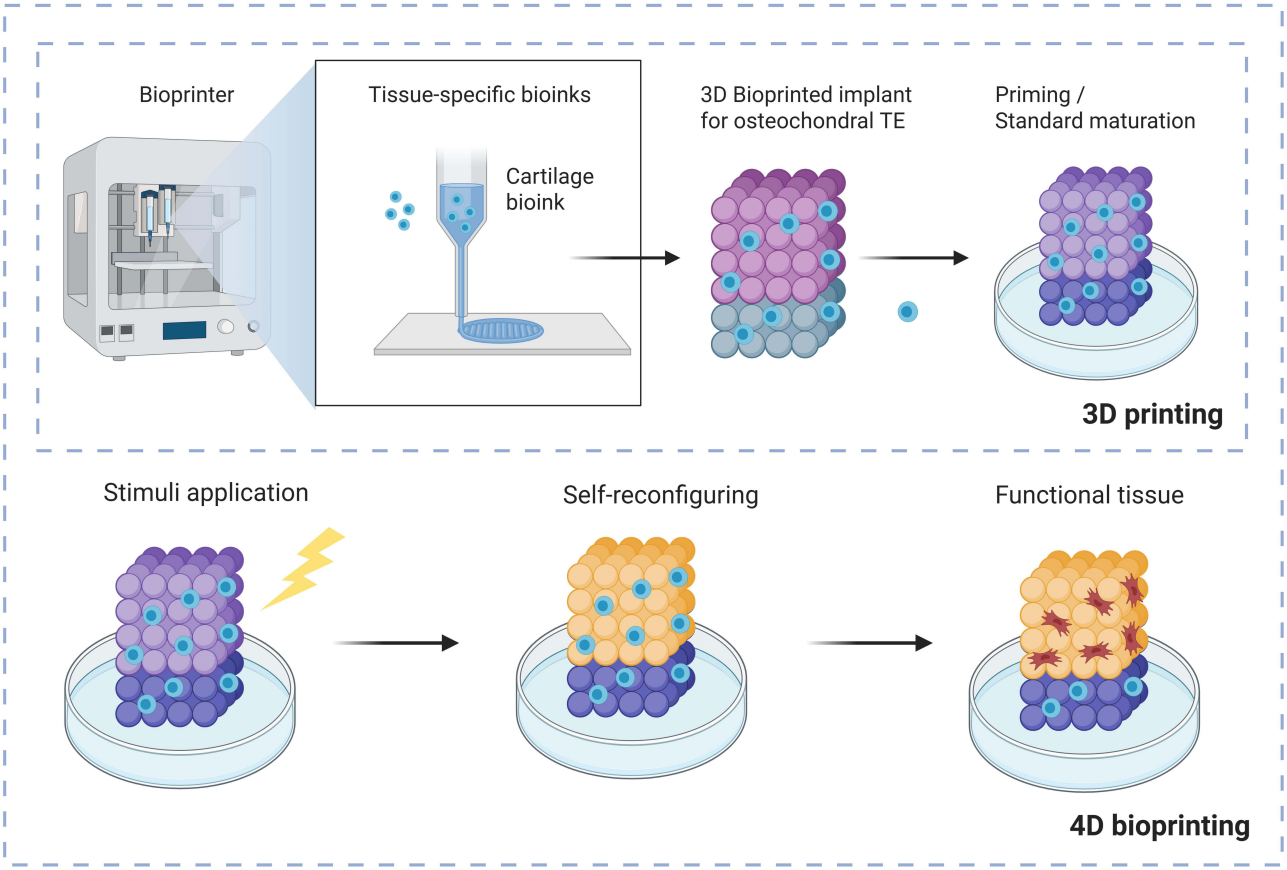

Figure 7 This schematic illustrates the workflow of 3D and 4D bioprinting for osteochondral tissue engineering. Tissue-specific bioinks, such as cartilage bioinks, are deposited using a bioprinter to fabricate three-dimensional bioprinted implants. The printed constructs undergo priming or standard maturation to support initial tissue development. Upon exposure to external stimuli, the constructs exhibit self-reconfiguration, leading to spatial and functional remodeling over time. This dynamic maturation process results in the formation of functional tissues, highlighting the transition from static 3D bioprinting to stimulus-responsive 4D bioprinting for the generation of complex, functional osteochondral tissues.

4. Artificial Intelligence and Big Data-Driven Design: Utilizing machine learning and computational models to analyze vast amounts of printing parameters, material properties, and tissue functional output data, reverse-engineering optimal printing strategies to accelerate R&D.
5. Focus on Clinical Translation Research: Promoting long-term (>1 year) repair studies in large animal models (eg, sheep, pigs) to systematically evaluate the safety, efficacy, and biomechanical function of printed cartilage organoids, while actively collaborating with regulatory agencies to establish product standards.

Conclusion

3D bioprinting is reshaping cartilage regenerative medicine. This review highlights advances in bioinks, printing technologies, and functionalization strategies, with three key insights: First, successful cartilage organoid construction requires balancing multiple factors; no single material or technique currently meets all demands of printability, mechanical strength, biocompatibility, and biomimicry. Hybrid materials, such as attapulgite–PVA composites, demonstrate synergistic optimization. Second, functional biomimicry goes beyond structural replication. The focus is on regulating biological processes, including immune responses (eg, macrophage polarization and NETs clearance), spatiotemporal delivery of growth factors, and dynamic mechanical cues to achieve stable hyaline cartilage without hypertrophy or fibrosis. Third, clinical translation remains a major bottleneck, requiring standardized protocols, long-term large-animal studies, and scalable GMP-compliant manufacturing.

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Looking ahead, the integration of smart materials (4D bioprinting), organoid-on-a-chip systems, and AI-driven optimization may shift the field from structural imitation to functional regeneration, enabling personalized cartilage repair.

Data Sharing Statement

This is a review article, and all relevant information is provided in the article.

Ethical Approval and Consent to Participate

This is a review paper and does not involve direct research on humans or animals.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

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Disclosure

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