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REVIEW ARTICLE

Application and prospects of chitosan-based 3D-printed scaffolds in the repair of osteochondral defects

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Abstract

Osteochondral defects, which involve injury to both the articular cartilage and the underlying subchondral bone, present a considerable therapeutic challenge due to cartilage's poor intrinsic capacity for regeneration and the intricate, gradient structure of the osteochondral junction. Tissue engineering offers a promising strategy for regenerating this biphasic tissue. Chitosan has attracted significant research interest due to its favorable biocompatibility, controlled degradability, natural antibacterial activity, and structural resemblance to endogenous glycosaminoglycans. Integrating chitosan with 3D printing allows the production of scaffolds with customizable structures, porosity, and mechanical properties tailored to patient needs. Moreover, chitosan can easily be blended with various natural polymers to develop composite bioinks that improve osteogenic and chondrogenic potential, thereby enhancing the functional performance of scaffolds. This review examines research literature spanning January 2020 to October 2025. Recent advances include the development of functionalized chitosan derivatives for improved printability and crosslinking, as well as the incorporation of cells and growth factors to create bioactive, cell-laden constructs. This review provides an extensive overview of the physicochemical and biological characteristics of chitosan pertinent to osteochondral regeneration, discusses diverse 3D printing strategies utilized to construct chitosan-based composite scaffolds, and emphasizes their demonstrated potential in improving cellular responses, stimulating bone and cartilage formation, supporting biomineralization, and achieving controlled delivery of bioactive agents. Finally, we discuss current challenges, such as optimizing scaffold degradation kinetics and vascularization, and future perspectives on the clinical translation of these innovative constructs for effective osteochondral regeneration.

Keywords: 3D-printed; Chitosan; Osteochondral defect; Scaffold.

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1. Introduction

Osteochondral defects, a common challenge in orthopedics, are often difficult to repair completely due to the considerable heterogeneity in the structure, composition, and mechanical properties of cartilage and subchondral bone tissue.[1,2] Once osteochondral injuries occur, failure to intervene promptly can easily lead to joint dysfunction and even the onset and progression of osteoarthritis.[3,4] Traditional clinical treatments can alleviate symptoms and promote tissue repair to some extent; however, they generally suffer from limitations such as inadequate mechanical properties of the repaired tissues and a tendency for regenerated tissues to consist predominantly of fibrocartilage. This makes it difficult to achieve long-term, stable functional reconstruction.[5,6] The inherent heterogeneity of cartilage and subchondral bone in their composition, mechanical properties, and biological requirements makes designing a single scaffold that supports the regeneration of both tissues highly complicated.[7,8]

In recent years, promising alternatives in tissue engineering and regenerative medicine have emerged through the integration of scaffolds, cells, and bioactive molecules to replicate natural tissue environments.[9-11] Among various scaffold materials, chitosan stands out as an ideal osteochondral scaffold material due to its excellent biocompatibility, degradability, and antimicrobial properties.[12] More importantly, its molecular structure is highly similar to the glycosaminoglycans (GAGs) in the extracellular matrix (ECM) of cartilage, facilitating chondrocyte adhesion, proliferation, and cartilage-specific matrix secretion.[13] Meanwhile, with the rapid advancement of 3D printing technologies, chitosan-based tissue engineering scaffolds can now be fabricated with enhanced precision and tailored to meet patient-specific requirements.[7,14,15] 3D printing not only enables the construction of complex porous structures but also allows the optimization of microenvironmental conditions such as cell migration, nutrient transport, and angiogenesis by adjusting pore size, interconnectivity, and hierarchical distribution.[16] Additionally, 3D printing can be combined with bioactive factor loading to achieve spatially controlled, multi-factor delivery, thereby regulating chondrogenic and osteogenic differentiation simultaneously. This approach more closely mimics the natural repair pattern of the osteochondral interface.[17] Based on these advantages, 3D-printed chitosan-based composite scaffolds exhibit significant promise for osteochondral defect repair and provide new insights and technical support for future clinical translation.

Research on chitosan-based 3D-printed scaffolds demonstrates substantial diversity in material formulations, reflecting the various strategies researchers adopt to address repair requirements across different levels of osteochondral tissue. Regarding printing techniques, distinct 3D printing processes have been selected based on the intended application. In addition, performance evaluation approaches differ markedly. While most studies include fundamental physicochemical characterization alongside biological assessment, the scope of evaluation varies considerably. Some investigations focus solely on *in vitro* cell experiments, whereas others extend to *in vivo* animal studies. Overall, the application of chitosan 3D-printed scaffolds in osteochondral tissue engineering is rapidly advancing, characterized by multiple technical developments, diverse application fields, and varying evaluation criteria. However, this rapid development has also led to notable fragmentation and inconsistencies within the field. Differences in material formulations, printing processes, crosslinking methods, structural designs, and performance assessments create challenges in directly comparing results and hinder the establishment of consensus on optimal strategies. Therefore, a comprehensive and systematic review is urgently needed to help researchers more readily identify the strengths and limitations of chitosan-based 3D-printed scaffolds. In this context, we also discuss current challenges and future directions for translating these innovative scaffold designs from laboratory research to clinical applications.

This review followed a predefined search strategy applied to PubMed and Web of Science. Searches spanned January 1, 2020, to October 1, 2025, and were limited to English-language publications. Keywords included "chitosan," "3D printing," and "cartilage/osteochondral defect." After de-duplication, two authors independently screened titles and abstracts against prespecified inclusion and exclusion criteria, followed by full-text assessment to ensure accuracy. In total, 116 studies were included. Inclusion criteria encompassed studies investigating chitosan-based 3D-printed scaffolds for osteochondral repair, cartilage defect repair, or bone regeneration, using either pure chitosan or chitosan-based composites. Exclusion criteria were studies that did not involve 3D-printed chitosan scaffolds, were unrelated to the topic, or addressed non-osteochondral tissue engineering without reporting cartilage or bone repair outcomes.

2. Advantages of 3D printing technology in osteochondral tissue engineering

2.1. The complexity of osteochondral repair and its traditional limitations

The osteochondral tissue structure exhibits a unique gradient arrangement, progressing from superficial hyaline cartilage to intermediate calcified cartilage and finally to deep subchondral bone. Each layer of osteochondral tissue demonstrates a continuous gradient change in cellular morphology, ECM composition, and mechanical properties (e.g., stiffness, compressive strength) (Figure 1A).[18,19] This complex anatomical structure severely limits the self-repair capacity of osteochondral tissue following injury. Traditional treatment methods such as microfracture, autologous chondrocyte transplantation, or osteochondral grafting, though widely used clinically (Figure 1B), have significant limitations. First, the repaired tissue is predominantly fibrocartilage rather than hyaline cartilage, resulting in inadequate mechanical properties. Additionally, these approaches face challenges, including limited donor availability, secondary injury, and poor interface integration.[20,21] More importantly, the bonding strength at the osteochondral interface is often insufficient, leading to suboptimal repair outcomes.[22] Consequently, designing comprehensive repair strategies that replicate the hierarchical architecture and functional gradients of native osteochondral tissue has become a major challenge in osteochondral tissue engineering.

2.2. High-precision bionic manufacturing using 3D printing technology

2.2.1. Precise construction of gradient structures

Three-dimensional printing techniques allow accurate regulation of scaffold pore dimensions, overall porosity, and spatial organization at both micro- and nanoscale levels. This capability enables the precise fabrication of architectures that replicate the mechanical and biological gradient transitions between cartilage and subchondral bone at the osteochondral interface (Figure 2A and C).[23,24] Specifically, 3D printing not only enables the compartmentalized deposition of multiple materials within a single scaffold but also supports the construction of pore size, density, and composition gradients through adjustable printing parameters. This facilitates the formation of biomimetic structures that combine mechanical stability with biological activity.[25] Moreover, this technology can integrate bioactive factors or cells to achieve spatially targeted, multi-factor release, thereby inducing bone marrow-derived mesenchymal stem cells (BMSCs) to undergo region-specific differentiation and promoting synergistic repair at the osteochondral interface (Figure 2B).[20,26] For example, one study utilized hydroxypropyl chitosan and oxidized chondroitin sulfate as base materials to fabricate a dual-layer scaffold via 3D printing technology for osteochondral defect repair.[27] The upper layer of chitosan and chondroitin sulfate supports chondrocyte proliferation, while the lower layer incorporates calcium phosphate to facilitate osteogenesis and angiogenesis. This 3D-printed scaffold achieves a compositional gradient between the bone and cartilage layers, effectively promoting osteochondral repair. 3D-printed chitosan composite scaffolds combine the printability, modifiability, and bioactivity of chitosan with dual-layer and gradient structural designs. The upper layer provides a cartilage-like hydrogel microenvironment, while the lower layer incorporates composite materials enriched with osteogenic factors to enhance osseointegration.[7,28,29] Through 3D printing, continuous interlayer transitions and personalized pore size and shape control are achieved,

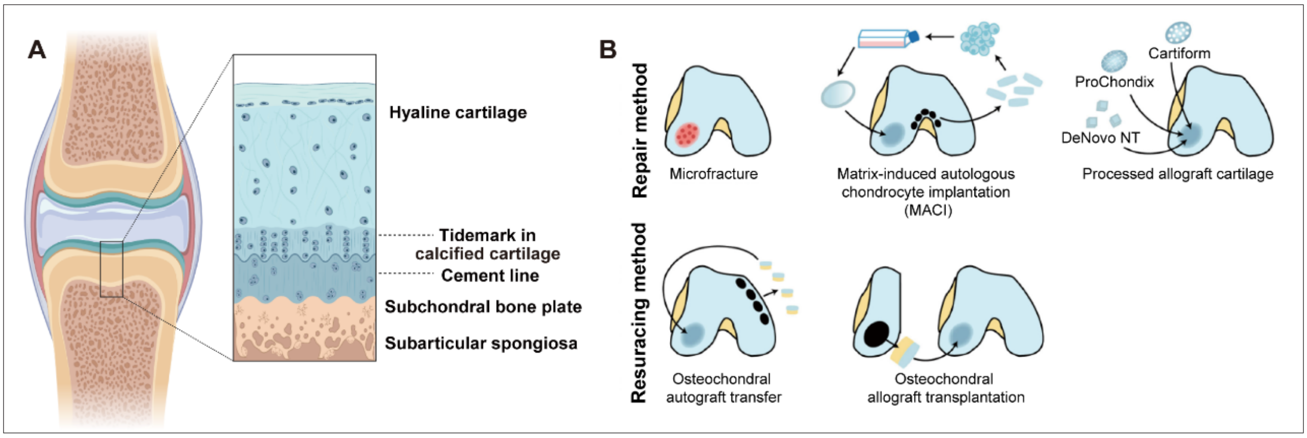

Figure 1. The structure of osteochondral tissue and treatment of osteochondral defects. (A) Schematic diagram of the structure of osteochondral tissue (Created in BioRender. der, b. (2025) https://BioRender.com/qfyzeqi). (B) Clinical treatment strategies for osteochondral defects. Reprinted with permission from Ref. [21] Copyright © 2019, Springer Nature Limited.

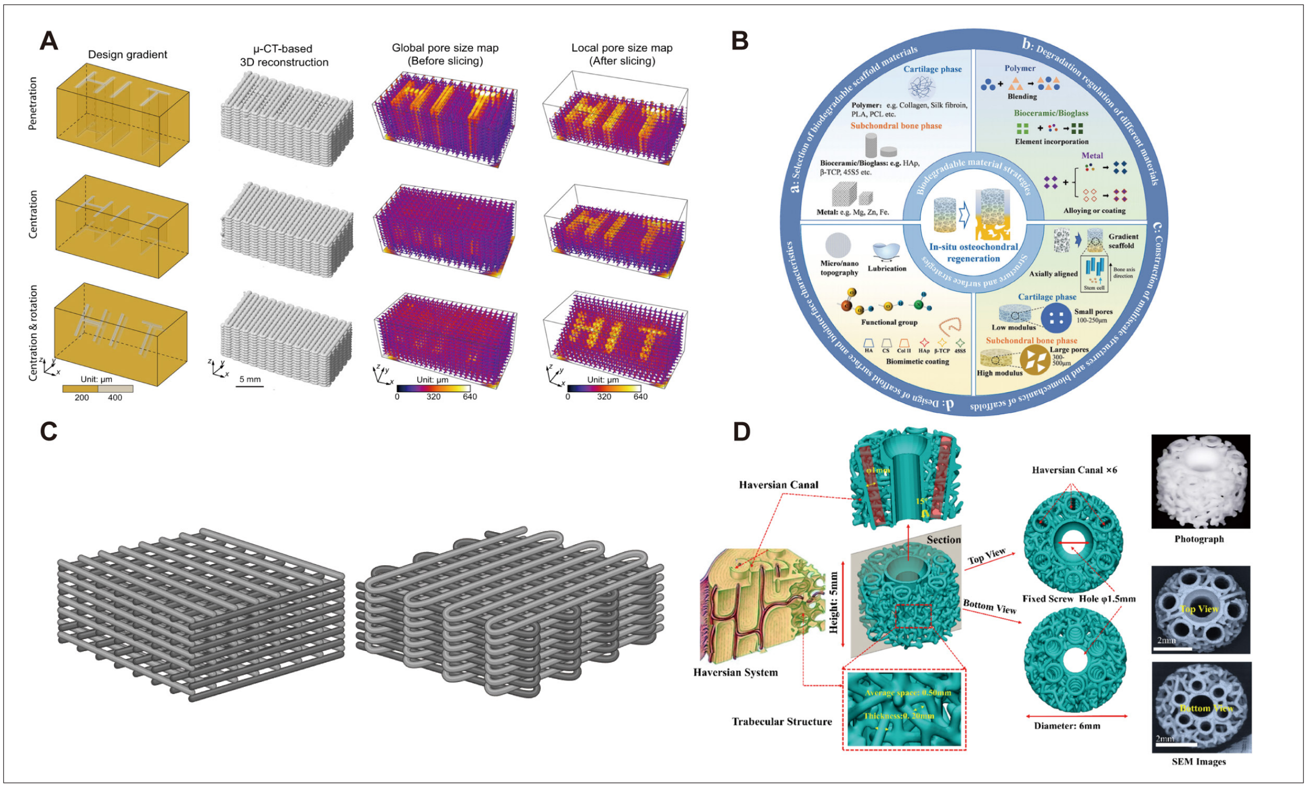

Figure 2. Structure, components, and parameters of 3D-printed osteochondral scaffolds. (A) Gradient structures with highly tunable pore size. Reprinted from Ref.[24] Copyright © 2024, The Authors. (B) Schematic diagram of key components for tissue engineering of osteochondral scaffolds. Reprinted with permission from Ref.[20] Copyright © 2024, Wiley-VCH GmbH. (C) Schematic diagram of the structure of a 3D-printed porous scaffold. Reprinted with permission from Ref.[20] Copyright © 2024, Wiley-VCH GmbH. (D) 3D design drawing of a bone scaffold incorporating Haversian canals and a trabecular porous structure. Reprinted from Ref.[30] Copyright © 2025, The Authors. Abbreviations: Col II: Collagen type II; CS: Chitosan; Fe: Iron; HAp: Hydroxyapatite; Mg: Magnesium; PCL: Polycaprolactone; PLA: Polylactic acid; SEM: Scanning electron microscopy; Zn: Zinc; β-TCP: Beta tricalcium phosphate; μ-CT: Micro-computed tomography.

ultimately delivering simultaneous benefits in three key aspects: interfacial integration, mechanical matching, and tissue-specific regeneration.

2.2.2. Precise regulation of surface microstructure

In osteochondral tissue engineering, scaffolds must not only possess overall mechanical stability and a macro-gradient structure but also provide a surface-level microenvironment that mimics the natural ECM. Such microstructural precision allows for effective modulation of cellular behaviors, including adhesion, migration, proliferation, and differentiation. Previous research has shown that the pore size, surface roughness, surface topology, and the spatial arrangement of scaffolds can significantly influence cellular morphological characteristics and signaling pathways, thereby determining the functional state of osteoblasts and chondrocytes.[31,32] Wang *et al.*[30] fabricated a dual-bionic ceramic scaffold combining Haversian canals and trabeculae via DLP 3D printing, incorporating surface microstructure modifications. The core mechanisms—structural orientation guidance, mechanical space maintenance, and bioactive microcrystals—offer a novel solution for precise shaping and efficient osteogenesis in maxillofacial bone defect repair (Figure 2D). Tang *et al.*[33] designed a 3D chitosan scaffold loaded with zinc oxide nanoparticles. Nanoparticle decoration enhanced the nanoscale topography and increased pore-wall roughness, thereby improving osteogenic potential. For chitosan-based 3D-printed scaffolds, pore-wall roughness, micro- and nanoparticle distribution, surface roughness, and overall topography can be regulated by adjusting printing parameters (such as filament diameter, layer thickness, and nozzle speed), applying post-processing techniques (including crosslinking, mineralization, and nanofiller modification), and incorporating composite materials. Integrating these design elements can significantly enhance the scaffold's potential for functional repair in osteochondral defects.

2.2.3. Personalized adaptation and surgical innovation

Three-dimensional printing technology offers personalized solutions for osteochondral repair by customizing scaffolds to match patient-specific shape, size, and internal structure based on computed tomography/magnetic resonance imaging data. This is particularly beneficial for irregular articular surface reconstruction. Robotic-assisted *in situ* printing systems can deposit bioink directly onto defect sites, using intraoperative 3D scanning to dynamically adjust printing paths for minimally invasive, precision repair.[34] The integration of 3D bioprinting with robot-assisted minimally invasive surgery provides a promising approach for accurately repairing localized cartilage lesions in the knee joint.[35] 3D-printed chitosan composite scaffolds achieve tailored mechanical properties and degradation profiles suitable for osteochondral defects through material compositing and modification. Their biomimetic structural designs ensure both anatomical conformity and compatibility with the cellular microenvironment, while optimized functional loading supports the sequential demands of osteochondral repair. This approach represents an ideal strategy for personalized treatment of osteochondral defects. With ongoing advancements in smart materials, artificial intelligence-assisted design, and 3D printing technology, 3D-printed chitosan-based composite scaffolds are expected to play an increasingly important role in precision and minimally invasive osteochondral tissue engineering.

3. Overview of chitosan

3.1. Structure

Chitosan is the only naturally occurring cationic polysaccharide and is widely found in the cells of lower plants, the cell walls of higher plants, and the shells of crustaceans. Its structure consists of randomly distributed N-acetylglucosamine and D-glucosamine units. The molecular structure of chitosan resembles certain polysaccharide repeating units found in articular cartilage, giving it properties similar to hyaluronic acid and GAGs within the ECM.[5] Therefore, chitosan can partially mimic the ECM of articular cartilage and promote cartilage tissue formation. Furthermore, the numerous hydroxyl and amino groups distributed along the chitosan molecular chain confer excellent hydrophilicity, biocompatibility, and chemical reactivity.[16,36,37] These functional groups also facilitate chemical modification, making chitosan readily compatible with composite applications with other materials. For example, Chen *et al.*[38] grafted the chondrogenic-promoting small-molecule drug kartogenin (KGN) onto chitosan to construct a cartilage repair hydrogel. *In vitro* and *in vivo* experiments confirmed that the KGN-functionalized chitosan hydrogel effectively promotes cartilage regeneration. In addition, the positive charges inherent to chitosan support its interaction with negatively charged cells and proteins. In summary, chitosan is a promising natural biomaterial for repairing cartilage defects.

3.2. Physicochemical properties

Chitosan exhibits poor solubility in water and alkaline media but readily dissolves in dilute acidic environments. Under acidic conditions, the amino groups in chitosan bind with hydrogen ions from the acid, forming positively charged ions that allow the chitosan molecules to dissolve.[39] This pH-dependent solubility enables its processing under mild conditions, creating opportunities for various 3D printing techniques.[40] Chitosan solutions display pronounced viscosity, a rheological property that offers unique advantages during 3D printing.[41] Compared with commonly used natural polymers such as sodium alginate and hyaluronic acid, hydrogen bonding and electrostatic interactions between chitosan molecules generate stronger cohesive forces. This enables chitosan to form continuous, uniform, and stable filaments during extrusion printing, reducing filament breakage and spreading. As a result, printing precision and repeatability are significantly enhanced.[42] Meanwhile, chitosan solutions maintain their designed morphology for a short period after deposition, providing a sufficient operational window for subsequent physical or chemical crosslinking. This characteristic is particularly crucial for achieving regionalized printing of cartilage and bone tissues.[43]

During 3D printing, the printability of the material is a crucial factor determining dimensional accuracy and structural stability.[18] An ideal bioink should possess suitable rheological properties to ensure smooth flow through the nozzle while rapidly maintaining its designed shape after deposition.[41,44] Due to its distinctive molecular configuration and favorable rheological behavior in solution, chitosan demonstrates excellent suitability for printing applications. On the one hand, chitosan solutions exhibit moderate viscoelasticity within a specific concentration range, enabling smooth extrusion under shear forces and ensuring continuity and consistency during the printing process.[42,45] On the other hand, their excellent interlayer adhesion significantly enhances the overall stability of printed structures, supporting the successful construction of complex 3D porous and gradient structures without collapse or deformation.[11]

3.3. Biological function

3.3.1. Excellent biocompatibility

Chitosan-based composite scaffolds play a crucial role in promoting cellular bioactivity due to their similarity to the natural ECM, supporting cell adhesion, proliferation, and differentiation.[12] They also demonstrate excellent biocompatibility and biodegradability in osteochondral tissue engineering.[13] Chitosan can be gradually degraded by ECM enzymes after implantation and ultimately absorbed or eliminated by the body, thus avoiding immune or toxic reactions associated with long-term implantation.[46] The incorporation of graphene into chitosan scaffolds enhances both mechanical integrity and biocompatibility. Studies have shown that 3D-bioprinted composite scaffolds composed of chitosan, gelatin, hyaluronic acid, and graphene effectively support the adhesion, proliferation, and growth of BMSCs.[15] Chang *et al.*[46] prepared a methyl acrylate–glycol chitosan bioink loaded with MG-63 cells and fabricated scaffolds using a photopolymerization-based 3D printing system. As shown in Figure 3A, *in vitro* experiments demonstrated that cell viability exceeded 92%, while the proliferation rate remained above 96%. These findings confirm the scaffold's excellent biocompatibility and highlight its strong potential for bone tissue regeneration.

3.3.2. Antibacterial activity

Osteochondral tissue engineering often involves trauma repair and implant surgery, where surgical sites are susceptible to bacterial infection, potentially leading to repair failure or delayed recovery.[47] Chitosan, as a natural antimicrobial material, therefore offers significant advantages for osteochondral repair. Its antibacterial activity mainly originates from its protonated amino groups ($-NH_2$), which interact electrostatically with negatively charged bacterial membranes, disrupting membrane integrity and function.[48] Modifying chitosan's molecular structure or combining it with other antimicrobial agents can further enhance its activity, especially against Gram-negative bacteria.[11,49] One study fabricated chitosan scaffolds via 3D printing and incorporated chitosan microspheres loaded with vancomycin and diflunisal to achieve coordinated control of bacterial infection for treating osteomyelitis (Figure 3B).[47] To further improve antibacterial efficacy, chitosan has been modified into quaternized chitosan (QCS) and combined with tricalcium phosphate (TCP) for 3D printing. When loaded with iron-doped barium titanate, tricobalt oxide, curcumin, and engineered cell membranes, this approach yields 3D-printed chitosan-based scaffolds containing magneto-ultrasonic dual-responsive nanoparticles (Figure 3C).[11] Both *in vitro* and *in vivo* studies have shown that these scaffolds effectively eradicate biofilms, exhibit immunoregulatory properties, and promote bone regeneration, offering a promising strategy for treating infected bone defects.

3.3.3. Cell proliferation and differentiation promotion

Chitosan facilitates the proliferation of osteoblasts and chondrocytes while inducing the differentiation of BMSCs, processes that are essential for effective osteochondral tissue regeneration.[50] Chitosan can bind to receptors on cell membranes, enhancing the affinity between cells and scaffolds.[51] It can also trigger various cellular signaling cascades by interacting with cell membrane receptors, including pathways like mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt). These signaling pathways are essential for regulating cellular processes such as proliferation, survival, and growth.[52] Polyethylene glycol diacrylate (PEGDA), a commonly used 3D printing material, lacks biocompatibility and osteogenic activity. However, by incorporating chitosan into the bioink, the scaffold's biocompatibility was significantly enhanced, indicating that it has the potential to promote cell proliferation.[14] Nguyen and colleagues[7] developed a 3D-printed bilayer scaffold specifically engineered to meet the distinct functional needs of cartilage and bone tissues. The cartilage component consisted of N, O-carboxymethyl chitosan combined with oxidized xanthan gum (OXG), designed to replicate the characteristics of the native ECM. The subchondral bone layer incorporated biphasic calcium phosphate (BCP) to enhance osteoconductivity. This bilayer printing approach achieved structural biomimicry, mechanical matching, and synergistic bioactivity, thereby improving the efficiency of osteochondral defect repair (Figure 3D).[7] Studies have demonstrated that chitosan-based materials stimulate the proliferation of bone marrow-derived osteoprogenitor cells and promote their maturation into functional osteoblasts through the upregulation of osteogenic gene expression.[53] Furthermore, chitosan significantly promotes bone formation by upregulating the expression of key genes, including *RUNX2*, *ALP*, and osteocalcin, in osteoblasts.[54] Chitosan also enhances the activity of the cartilage-inducing growth factor transforming growth factor-beta (TGF-β), thereby promoting cartilage formation.[55]

3.3.4. Loading of bioactive molecules

In osteochondral tissue engineering, scaffolds must not only provide structural support but also facilitate cellular proliferation, differentiation, and tissue regeneration. In recent years, bioactive agents—including metal ions, growth factors, and pharmacological compounds—have attracted considerable research interest due to their pivotal functions in regulating cell behavior and promoting tissue repair and regeneration. Incorporating these bioactive components into scaffold systems can significantly enhance cellular activity, thereby expediting the processes of osteochondral healing and regeneration.[56] Chitosan scaffolds, known for their superior biocompatibility, degradability, and adjustable structure, serve as an ideal platform for delivering bioactive factors. Additionally, 3D printing technology provides innovative solutions for the precise incorporation of these factors into the scaffolds. A 3D-printed hydrogel was constructed using recombinant collagen, chitosan, nano-clay, and KGN-loaded nanoparticles through electrostatic interactions and photopolymerization (Figure 3E). The controlled release of KGN from the scaffold promoted the chondrogenic differentiation of BMSCs, markedly increasing the expression of collagen type II and GAGs.[17] In a related study, a cartilage regeneration platform was developed using a chitosan hydrogel combined with a 3D-printed polycaprolactone (PCL) scaffold incorporating tetrahedral framework nucleic acids (TFNAs) and mesenchymal stem cells (MSCs). TFNAs, a class of DNA nanomaterials capable of optimizing the regenerative microenvironment, were introduced into MSCs to stimulate their proliferation and chondrogenic differentiation, thereby enhancing cartilage formation and improving the repair of cartilage lesions.[57]

3.4. Advantages compared with other 3D printing materials

In the field of osteochondral tissue engineering, 3D printing has emerged as a versatile platform for fabricating scaffolds or bioinks with customized architecture, controlled porosity, and spatial distribution of cells and biomolecules. The selection of the printing material is a critical determinant of the scaffold's mechanical properties, degradation profile, biocompatibility, cell-adhesive behavior, and ultimately regenerative outcome. Here, we compare major classes of materials used in 3D printing for osteochondral regeneration and then highlight the specific advantages of chitosan-based systems.

Natural polymers include collagen, gelatin, alginates, hyaluronic acid, chitosan, and others.[58] Their advantages lie in excellent biocompatibility, similarity to ECM components, and typically good cell adhesion and bioactivity.[59] However, they also share significant limitations, such as generally low mechanical strength, rapid degradation, poor printability, and insufficient mechanical stability for load-bearing bone applications. Synthetic polymers include PCL, poly (lactic-co-glycolic acid), polyethylene glycol (PEG), and polyetheretherketone.[60] These materials typically offer good mechanical property tunability, high structural strength, slower and controllable degradation characteristics, and are widely used in 3D printing.[59] However, they generally lack inherent bioactivity, produce acidic degradation products that hinder tissue repair, fail to mimic ECM signaling, and exhibit poor cell infiltration or vascular integration.[61] Bioceramic composites include hydroxyapatite, β-TCP, and bioactive glass. They exhibit excellent osteogenic properties, superior bioactivity, suitability for bone regeneration, and typically high mechanical strength.[20] However, they are relatively brittle with poor flexibility, which poses challenges when printing complex geometries or gradient structures, especially in softer cartilage regions.[62] Additionally, they may degrade too slowly or fail to match the mechanical modulus of cartilage.[63]

Recent studies indicate that chitosan-based 3D printed composite scaffolds demonstrate significant potential in osteochondral tissue engineering compared to other 3D printing materials, primarily due to the following aspects. First, chitosan can be readily modified or blended with other polymers to form bioinks with tunable rheological properties.[7,33] Second, chitosan exerts beneficial effects on both osteogenesis and chondrogenesis, making it highly suitable for osteochondral scaffold design, particularly under the regenerative demands of these dual tissue types.[64,65] Chitosan readily forms covalent bonds with other bioactive molecules and can be combined with other materials to enhance its mechanical and biological properties.[38] Unlike certain synthetic polymers that may degrade into acidic byproducts, chitosan undergoes a mild degradation process, yielding harmless products.[58] This is particularly important for tissue engineering. In cartilage regeneration, chitosan's ability to mimic the ECM and support GAG and type II collagen production is paramount. Finally, due to structural and functional differences between cartilage and subchondral bone, osteochondral repair scaffolds often require gradient compositions or zoned architectures.[7,16] Chitosan's flexibility and compatibility with blending other materials make it well-suited for such designs. Therefore, compared to existing biomaterials, chitosan emerges as a highly promising 3D-printable material for osteochondral regeneration strategies due to its biofunctionality, adaptability, and suitability for gradient designs.

4. Mechanism of chitosan in promoting osteochondral repair

Chitosan has been widely investigated for its potential in promoting cartilage repair and bone tissue regeneration. Its biological activity is not only reflected in its excellent biocompatibility and degradability but also in its regulatory effects on cells. Particularly during osteochondral repair, chitosan can promote cartilage and bone regeneration through multiple molecular mechanisms (Figure 4).

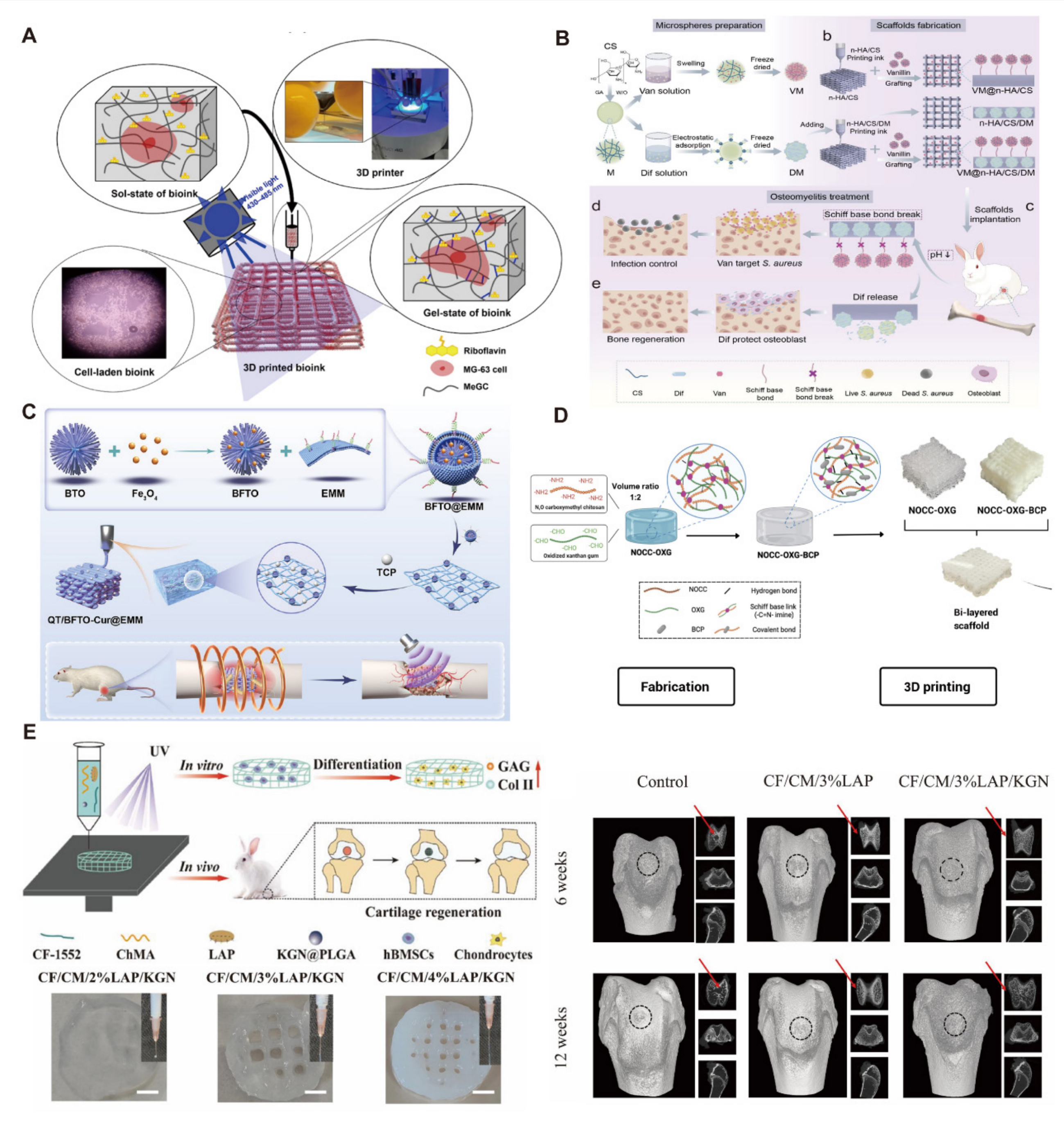

Figure 3. Utilization of 3D-printed chitosan-based composite scaffolds in the field of osteochondral tissue engineering. (A) Schematic representation of the fabrication process for 3D bioprinting using methyl acrylate-glycol chitosan (MeGC)-based bioink. Reprinted with permission from Ref. [46]. Copyright © 2025, Elsevier. (B) The 3D-printed chitosan scaffold loaded with vancomycin-chitosan microspheres (VM) is used for the treatment of osteomyelitis. Reprinted with permission from Ref. [47]. Copyright © 2025, Elsevier. (C) Quaternized chitosan combined with dual-responsive nanoparticles for the treatment of infected bone defects. Reprinted with permission from Ref. [11]. Copyright © 2024, American Chemical Society. (D) 3D-printed chitosan bilayer scaffolds for osteochondral defects. Reprinted from Ref. [7]. Copyright © 2025, Elsevier. (E) Chitosan hydrogels loaded with kartogenin (KGN) at different nanoclay concentrations promote cartilage repair. Reprinted from Ref. [17]. Copyright © 2024, The Authors. Abbreviations: BCP: Biphasic calcium phosphate; BFTO: Iron-doped barium titanate; BTO: Barium titanate; CF: Recombinant collagen CF-1552; Col II: Collagen type II; CM: chitosan methacrylate (ChMA); CS: Chitosan; Cur: Curcumin; DM: Diflunisal-loaded chitosan microsphere; EMM: Engineered mesenchymal stem cell membranes; GA: Glutaraldehyde; GAG: Glycosaminoglycans; hBMSCs: Human bone marrow-derived mesenchymal stem cells; LAP: Lithium phenyl-2,4,6-trimethylbenzoylphosphinate; NOCC: N, O-carboxymethyl chitosan; n-HA: Nano-hydroxyapatite; OXG: Oxidized xanthan gum; PLGA: Poly (lactic-co-glycolic acid); QT: quaternized chitosan (QCS); *S. aureus*: *Staphylococcus aureus*; TCP: Tricalcium phosphate; UV: Ultraviolet.

4.1. Molecular mechanisms promoting cartilage repair

Chitosan enhances the adhesion of MSCs by facilitating electrostatic interactions between its NH_2 groups and the negatively charged components of the MSC membrane, thereby improving the scaffold's overall cell-binding efficiency.[66] By interacting with specific receptors on the chondrocyte surface, chitosan can trigger various signaling cascades, such as the PI3K/Akt and MAPK pathways, thereby enhancing chondrocyte proliferation and differentiation and promoting the regeneration of injured cartilage tissue.[64]

Chitosan facilitates cellular adhesion and proliferation through electrostatic interactions between its positively charged functional groups and the GAGs present in the ECM.[5] The Wnt/β-catenin signaling pathway is pivotal in regulating the lineage commitment of MSCs. When Wnt/β-catenin activity is moderately stimulated, MSCs exhibit elevated expression of pluripotency-associated genes such as *Oct4*, *Sox2*, and *Nanog*, thereby enhancing their capacity for chondrogenic differentiation.[66] In addition, chitosan influences the Notch signaling cascade, which is essential for preserving MSC stemness and preventing premature differentiation.[67] Chitosan can also modulate the hypoxia-inducible factor-1 alpha pathway, which enables MSCs to maintain their stemness under hypoxic conditions and enhances the chondrogenic differentiation capacity of BMSCs.[68] The structure of chitosan resembles that of natural GAGs in cartilage tissue, providing chondrocytes with a favorable microenvironment that helps maintain the cartilage phenotype.[65]

Chitosan is also instrumental in regulating macrophage polarization, a key process in tissue repair. Chitosan facilitates the transition of macrophages from the pro-inflammatory M1 state to the regenerative M2 phenotype by modulating receptor-mediated signaling and cytokine secretion.[69] Interleukin (IL)-10, TGF-β, and insulin-like growth factor 1 (IGF-1) released by M2 macrophages can suppress excessive inflammatory responses and promote the proliferation and differentiation of chondrocytes, thereby contributing to cartilage regeneration.[70] Additionally, chitosan can promote chondrocyte proliferation and the chondrogenic differentiation of MSCs by modulating the M2 macrophage phenotype and activating the TGF-β receptor signaling pathway.[4]

4.2. Molecular mechanisms promoting osteogenesis

Chitosan not only promotes cartilage repair by regulating BMSCs and chondrocytes but also exerts regulatory effects on bone-formation-related cells such as osteoblasts and osteoclasts. These cells play crucial roles in osteochondral regeneration, and chitosan can influence their functions through multiple mechanisms.

4.2.1. Regulation of osteoblasts

Osteoblasts serve as the principal cells responsible for bone formation and mineral deposition. Chitosan influences osteoblast behavior by modulating their proliferation, differentiation, and mineralization processes. It enhances osteogenic differentiation by upregulating key transcription factors, including *Runx2* and *Osterix*, which are essential for osteoblast maturation and function.[71] The activation of these genes promotes osteoblast maturation and enhances bone matrix synthesis. Chitosan can also promote osteoblast differentiation through the Wnt/β-catenin signaling pathway. This pathway is essential for osteoblast development, and chitosan enhances the mineralization capacity of osteoblasts via its activation.[72] Chitosan enhances the expression of alkaline phosphatase (ALP) and osteocalcin, which are hallmark molecules involved in osteoblast mineralization.[54] Furthermore, when combined with bone-inducing materials such as hydroxyapatite and calcium phosphate, chitosan can further enhance bone mineralization.[7,14]

4.2.2. Regulation of osteoclasts

Osteoclasts are essential for bone remodeling and resorption. Chitosan modulates osteoclast function through several mechanisms. It can inhibit osteoclast differentiation and activity by promoting osteoblast secretion of osteoprotegerin, which competitively binds receptor activator of NF-kappaB ligand (RANKL) and prevents its interaction with the RANK receptor on osteoclast precursor cells.[73] Because RANKL is a critical regulator of osteoclast differentiation, its inhibition reduces bone resorption and supports bone regeneration. A composite scaffold integrating cerium-doped nano-hydroxyapatite with chitosan demonstrates superior osteoinductive potential. This hybrid structure enhances MSC adhesion and proliferation, elevates the expression of osteogenic markers such as *Runx2* and osteopontin, and concurrently suppresses osteoclast differentiation.[73,74] Chitosan also reduces osteoclast activity and bone resorption by inhibiting the secretion of inflammatory cytokines. Inflammatory cytokines such as tumor necrosis factor alpha and IL-6 typically activate osteoclasts by enhancing RANKL expression, making chitosan's anti-inflammatory effects beneficial for bone repair.[75]

Chitosan promotes osteochondral regeneration through multiple mechanisms, encompassing cartilage repair, osteoblast differentiation, bone mineralization, and immune regulation. In cartilage repair, chitosan is essential for stimulating chondrocyte proliferation and differentiation while enhancing cartilage matrix synthesis. Regarding the molecular mechanisms involving osteoblasts, osteoclasts, and macrophages, chitosan promotes bone tissue repair by regulating relevant gene expression, inhibiting bone resorption, and modulating immune responses. Future optimization of chitosan's biological activity, particularly through composite use with other biomaterials, holds promising prospects for its application in osteochondral tissue engineering.

5. Applications of modified chitosan materials in 3D printing technology

To further optimize the performance of chitosan-based 3D-printed scaffolds in osteochondral tissue engineering, various modification techniques are commonly employed to enhance their mechanical properties, degradation characteristics, and bioactivity. Modified chitosan materials can better meet clinical demands, demonstrating particular advantages in osteochondral tissue repair. The following sections detail the applications of several commonly used modified chitosan 3D-printed materials in osteochondral tissue engineering.

5.1. Carboxymethyl chitosan

Carboxymethyl chitosan (CMCS) is synthesized through the chemical modification of hydroxyl and amino functional groups on the chitosan backbone using carboxymethylation reagents. This material degrades during tissue regeneration with low toxicity and minimal inflammatory response, making it widely used in tissue engineering.[36] A study employed 3D printing to construct a bilayer scaffold, incorporating a CMCS–OXG hydrogel as the cartilage layer and BCP as the underlying bone layer. This structure mimics natural osteochondral tissue and promotes osteochondral regeneration (Figure 5A and 5B).[7] Wang *et al.*[12] enhanced hydrogel cross-linking, mineralization, and degradation rates tailored to the osteogenic cycle by adjusting the CMCS concentration in the hydrogel scaffold. This adjustment imparted a bioactive, microscale porous architecture to the material. *In vitro* experiments confirmed the scaffold's osteogenic potential, revealing that CMCS effectively enhances BMSC adhesion, proliferation, and differentiation.[12]

5.2. Quaternized chitosan

Quaternized chitosan inhibits bacterial metabolic activity by interfering with the tricarboxylic acid cycle, which disrupts cell wall formation and increases cell membrane permeability, thereby exerting strong antibacterial effects.[16,37] Additionally, QCS stimulates macrophages to secrete pro-inflammatory factors, inducing M1 polarization and enhancing antibacterial efficacy, with notable advantages in promoting bone regeneration in infected bone defects.[37] In one study, biologically active iron-doped barium titanate nanoparticles were synthesized and integrated into a composite of QCS and TCP, resulting in a bioink designed for 3D printing of antibacterial bone regeneration scaffolds. *In vivo* experiments demonstrated that these scaffolds markedly improved the repair of infected bone defects and highlighted macrophage-targeted modulation as an effective approach for simultaneously combating infection and enhancing bone regeneration.[11] Kang *et al.*[29] developed a composite scaffold (dGQH) consisting of a decellularized ECM, gelatin, QCS, and nano-hydroxyapatite. The dGQH20 scaffold exhibited excellent antibacterial properties (94.90 ± 2.44% inhibition of *Escherichia coli* and 95.41 ± 2.65% inhibition of

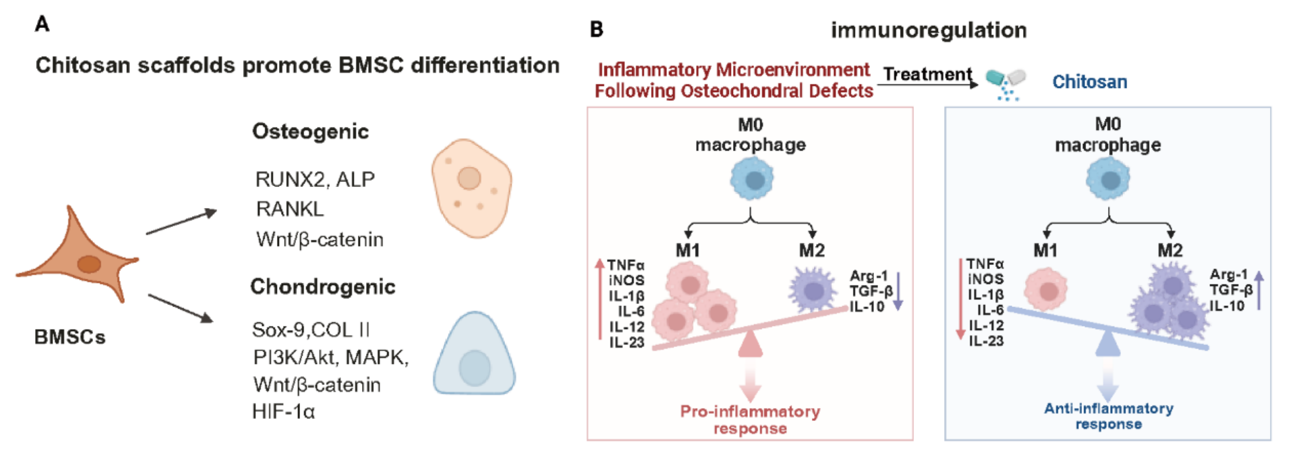

Figure 4. The mechanisms through which chitosan biomaterials influence the function of mesenchymal stem cells and macrophages (*Created in BioRender. der, b. (2025) https://BioRender.com/qfyzeqi*). Abbreviations: Arg-1: Arginase-1; BMSCs: Bone marrow-derived mesenchymal stem cells; IL: Interleukin; iNOS: Inducible nitric oxide synthase; TGF-β: Transforming growth factor-beta; TNFα: Tumor necrosis factor alpha

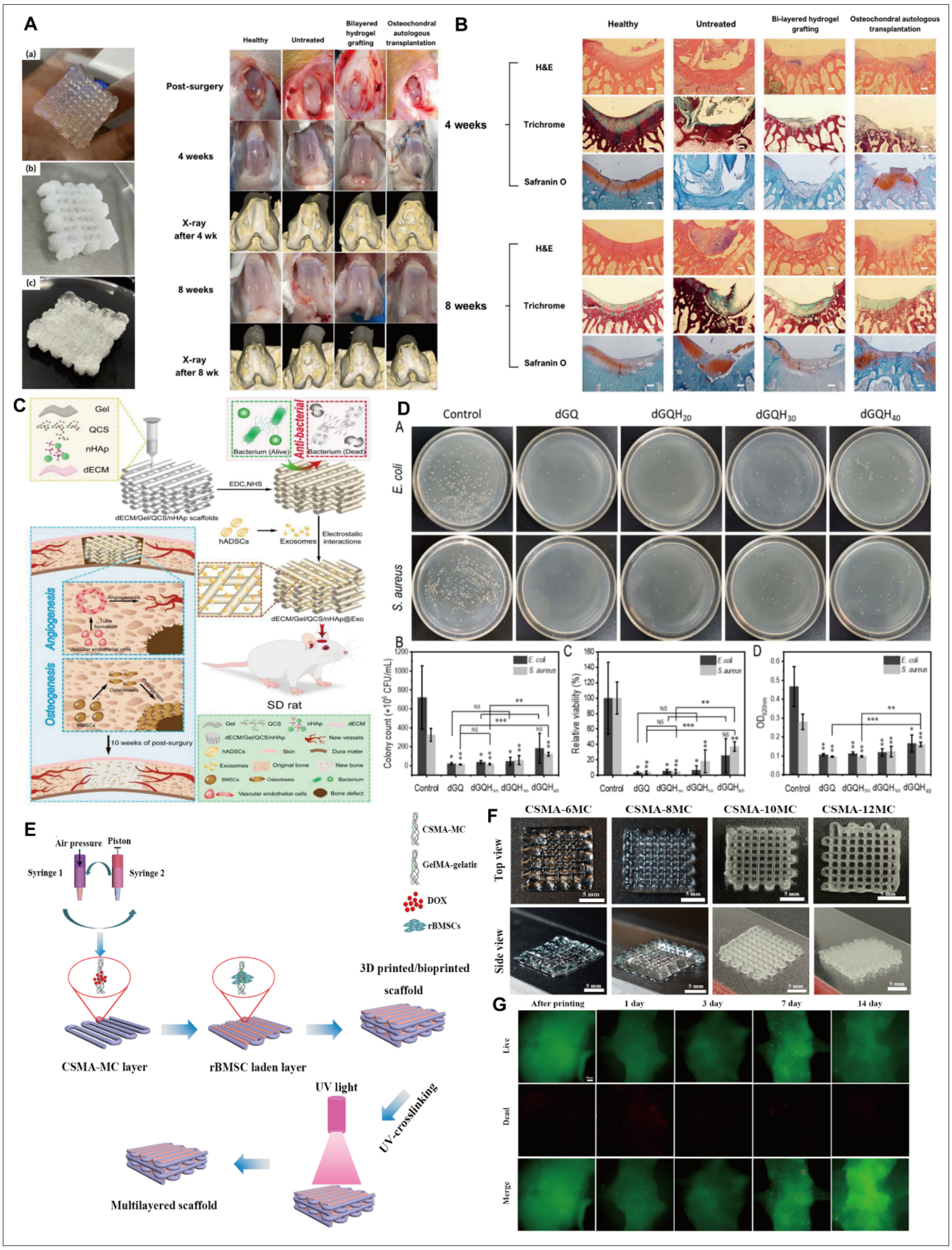

Figure 5. Application of modified chitosan 3D-printed scaffolds in osteochondral defects. (A) Schematic diagram of the bilayer printed scaffold and analysis of its efficacy in repairing osteochondral defects. Reprinted from Ref. [7]. Copyright © 2025, The Authors. (B) Histological analysis of repaired osteochondral tissue. Reprinted from Ref. [7]. Copyright © 2025, The Authors. (C) Schematic illustration of the fabrication process for the decellularized extracellular matrix (dECM)/gelatin (Gel)/quaternized chitosan (QCS)/nano-hydroxyapatite (nHAp) composite scaffold. Reprinted with permission from Ref. [29]. Copyright © 2023, IOP Publishing Ltd. (D) Antibacterial properties of QCS scaffolds. Reprinted with permission from Ref. [29]. Copyright © 2023, IOP Publishing Ltd. (E) Schematic depicting the construction of a stratified hydrogel scaffold incorporating both therapeutic agents and living cells. Reprinted from Ref. [77]. Copyright © 2024, The Authors. (F) Physical images of 3D-printed hydrogels containing different components. Reprinted from Ref. [77]. Copyright © 2025, The Authors. (G) Biocompatibility of bioprinted cell-loaded scaffolds. Reprinted from Ref. [77]. Copyright © 2025, The Authors. Abbreviations: CSMA: Chitosan methacrylate; dGQ: 3D hybrid scaffold composed of dECM, Gel, and QCS; dGQH: 3D hybrid scaffold composed of dECM, Gel, QCS, and nHAp; DOX: Doxorubicin; EDC: Ethyldimethylaminopropyl Carbodiimide; *E. coli*: *Escherichia coli*; GelMA: Gelatin methacrylate; hADSCs: Human adipose derived stem cells; MC: Methylcellulose; NHS: N-Hydroxysuccinimide; rBMSC: Rat bone marrow mesenchymal stem cell; SD: Sprague–Dawley; *S. aureus*: *Staphylococcus aureus*; UV: Ultraviolet.

Staphylococcus aureus), while also significantly promoting osteogenesis and angiogenesis at bone defect sites (Figure 5C and 5D).

5.3. Chitosan methacrylate

Chitosan methacrylate (CSMA) enhances crosslinking and structural stability by introducing methacrylate groups into the chitosan backbone. During 3D printing, CSMA exhibits superior shape retention and mechanical stability, making it suitable for fabricating osteochondral repair scaffolds that require high strength and durability.[76] Customized mechanical properties can be achieved by adjusting the methacrylation degree, solution concentration, and crosslinking conditions to meet the mechanical demands of osteochondral structures.[46] A study employed 3D printing to fabricate hydrogel scaffolds composed of CSMA and methylcellulose. The photopolymerized porous scaffolds exhibited excellent biocompatibility and mechanical properties, along with controllable drug release capabilities (Figure 5E–5G).[77] Researchers have also modified CSMA by grafting KGN to functionalize it. Hydrolysis of the amide bonds in the hydrogel enables continuous KGN release, promoting cartilage repair.[78] Additional components can also be incorporated into bioinks to achieve bone and cartilage regeneration. Zhang *et al.*[17] prepared photopolymerizable bioink using CSMA loaded with KGN nanoparticles to promote cartilage tissue regeneration. Additionally, cells can be incorporated into 3D-printed hydrogels to construct osteochondral scaffolds. Liu *et al.*[79] prepared a bioink by blending gelatin methacrylate with CSMA and encapsulating BMSCs to print a cartilage scaffold. Cell viability within the scaffold reached 91.1%, and the construct supported cartilage differentiation. Transplantation of the bioprinted hydrogel loaded with BMSCs into cartilage defects resulted in effective hyaline cartilage repair.[79]

Modified chitosan scaffolds demonstrate significant advantages in 3D printing technology. By introducing specific functional groups, the water solubility, antibacterial properties, and mechanical strength of chitosan are enhanced. Overall, these functional modifications improve the comprehensive performance of the scaffolds, offering a superior solution for osteochondral tissue engineering.

6. Limitations of chitosan composite scaffolds

Although the properties of chitosan and its derivatives have been described in detail, inherent limitations still exist that impact clinical translation. First, chitosan possesses several limitations in its physicochemical properties. Native chitosan is virtually insoluble under neutral and alkaline conditions, with its solubility and protonation strongly dependent on pH.[80] This results in restricted swelling, stability, and gelation windows under physiological conditions. Additionally, variations in source, degree of deacetylation, and molecular weight distribution lead to batch-to-batch inconsistencies and reproducibility issues.

Second, pure chitosan hydrogel scaffolds exhibit insufficient compressive strength, fatigue resistance, and shear resistance. Ionic crosslinking of chitosan provides weak structural stability under mechanical load, whereas photo-crosslinked derivatives such as CSMA require photoinitiators and ultraviolet or visible light exposure, which may introduce cytotoxic effects depending on dose and exposure duration.[78] Consequently, achieving a balance between printability, stability, and cytocompatibility remains a major technical bottleneck.

Furthermore, chitosan is degraded primarily via lysozyme-mediated enzymolysis and dissolution, resulting in unpredictable degradation kinetics.[81] Excessively rapid degradation may lead to scaffold collapse before tissue regeneration, whereas slow degradation can hinder new matrix deposition. The degradation rate is highly sensitive to the degree of deacetylation and polymer sequence, which are difficult to precisely regulate during synthesis. Chitosan is also highly susceptible to molecular degradation during sterilization. γ- or β-irradiation significantly reduces its molecular weight, altering viscosity, color, and mechanical strength, whereas ethylene oxide sterilization may leave cytotoxic residues.[82] These sensitivities complicate large-scale manufacturing, quality assurance, and regulatory compliance for clinical applications. Although layered or gradient scaffolds have shown encouraging results *in vitro* and in preclinical studies, complete integration between bone and cartilage compartments remains a key challenge.[51] Current constructs often exhibit inadequate interfacial strength, insufficient lubrication, and suboptimal fatigue resistance under cyclic joint loading. Long-term performance comparable to native osteochondral tissue has yet to be demonstrated.

Functional derivatives such as CMCS or QCS improve solubility, processability, and antibacterial activity; however, these modifications may reduce stiffness, alter charge density, or induce undesired cellular stress responses.[81] Similarly, photopolymerizable derivatives rely on external initiators, raising potential concerns regarding biocompatibility and reproducibility.

The current limitations and corresponding solutions are summarized in Table 1.

7. 3D printing technology based on chitosan-based composite scaffolds

In the field of osteochondral repair, various 3D printing technologies are employed to prepare scaffold materials, each with its own advantages and disadvantages. This section provides a comprehensive overview of the advantages, disadvantages, and future directions of different 3D printing methods combined with chitosan-based composite bioinks (Table 2; Figure 6). Furthermore, bioinks are crucial for the successful printing of tissue structures. Different 3D printing technologies impose distinct requirements on bioinks based on their underlying principles. Therefore, we also summarize the application scenarios for chitosan-based 3D bioinks.

7.1. Extrusion bioprinting

Extrusion-based bioprinting (EBP) employs a highly regulated dispensing mechanism to sequentially deposit bioinks, enabling the construction of complex 3D architectures (Figure 6A and 6B). The key to this technology lies in material extrusion, typically achieved by heating or applying mechanical pressure to force the bioink—usually composed of cells and biopolymers—through a nozzle, depositing it at predetermined locations.[91] Compared to other bioprinting techniques, EBP offers higher material compatibility, a larger printing range, and lower costs. Nguyen *et al*.[7] prepared hydrogels of CMCS and OXG using 3D printing technology to fabricate a bilayer osteochondral hydrogel scaffold. A BCP layer was added to the lower layer to induce osteogenesis. The cartilage layer was printed at a thickness of 1 mm, while the subchondral bone layer was printed at 2 mm. A porosity of 30% was set, which was demonstrated to be conducive to cell ingrowth and proliferation.[7] By modifying chitosan with double bonds and combining it with recombinant collagen and nano-clay to prepare a photopolymerizable bioink, a small-molecule drug (KGN) was loaded to promote cartilage differentiation. *In vivo* assessment revealed that scaffolds engineered via EBP successfully supported the regeneration of native cartilage tissues.[17]

However, EBP suffers from a narrow rheological window, leading to shear-induced cell damage, nozzle clogging, and limited resolution.[84] The ionic crosslinking typically used in chitosan systems provides poor long-term stability and nonuniform gelation, while weak interlayer bonding and low wear resistance restrict mechanical performance under joint loading.[92] Additionally, native chitosan exhibits pH-dependent solubility and limited cell adhesion, necessitating blending or modification.[93] Future progress will rely on multimodal crosslinking, microgel-reinforced formulations, and nanofiller incorporation to enhance mechanical integrity and bioactivity. Coaxial or microfluidic extrusion and 4D-printing strategies are expected to improve structural gradients, nutrient diffusion, and dynamic tissue integration, advancing chitosan-based bioinks toward functional osteochondral regeneration.

7.2. Stereolithography

Stereolithography (SLA) uses laser or ultraviolet light to cure photopolymer resin layer by layer, forming 3D

Table 1. Summary of major limitations of chitosan-based composite scaffolds and derivatives for osteochondral repair and common improvement strategies

Limitation	Manifestations	Improvement strategy	Ref.
pH-dependent solubility; batch variability	Difficult gelation/stability at physiological pH; inconsistent printability and biological outcomes	Use of water-soluble derivatives (CMCS, QCS); buffer optimization; control of MW	80
Insufficient mechanical properties	Low load-bearing capacity and poor boundary lubrication on cartilage surfaces	Composite or double-network designs; layered structures	83,84
Narrow crosslinking; photoinitiator cytotoxicity	Ionically crosslinked CS weak under load; photoinitiators may induce cell stress/toxicity	Visible-light initiators; optimized irradiation; hybrid chemical–physical crosslinking	85,86
Degradation-rate incompatibility	Rapid dissolution or enzyme-mediated degradation outpaces new tissue formation	Tuning MW; enzyme-responsive systems; mineral-reinforced composites for bone layers	87
Sterilization sensitivity	γ-/β-irradiation lowers molecular weight (MW), altering viscosity and mechanical stability	Validated irradiation doses; non-EtO sterilization; post-sterilization quality control	82,88
Poor interface integration	Weaker interface bonding; limited fatigue/lubrication matching under long-term cyclic loading	Gradient or bilayered scaffolds; tribological validation under simulated joint	51,89
Trade-offs of charged/soluble derivatives	QCS or carboxymethyl CS improves solubility but reduces stiffness or alters cell behavior	Control charge density; combine with inorganic fillers; low-toxicity initiator systems	81,90

Abbreviations: CMCS: Carboxymethyl chitosan; CS: Chitosan; EtO: Ethylene oxide; QCS: Quaternized chitosan.

objects. Compared to other 3D printing methods, SLA offers significant advantages in resolution and precision, enabling the creation of finer structures suitable for biomedical applications.[96] In osteochondral tissue engineering, the high-precision forming capability of SLA aids in reconstructing complex osteochondral structures and intricate microenvironments, providing an ideal scaffold for cell growth and differentiation. In this context, SLA printing combined with chitosan-based composite bioinks demonstrates distinct advantages in applications requiring microscale precision and surface fidelity rather than bulk mechanical strength. Its superior resolution makes it particularly suitable for fabricating cartilage-like zones, interfacial gradient layers, and microstructured templates that replicate the hierarchical organization of native cartilage. Conversely, SLA remains less suited for load-bearing subchondral scaffolds, where EBP or fused deposition modeling (FDM) techniques provide better mechanical stability. One study utilized SLA technology to construct a bilayer biomimetic periosteum. The upper layer consisted of a high-mechanical-property fiber layer made of PEGDA/chitosan/TCP, which prevented muscle compression and soft tissue invasion. The lower layer comprised a gelatin methacrylate/PEGDA/ammonium molybdate cambium layer. When combined with 808 nm near-infrared photothermal effects, this layer promoted MC3T3-E1 cell proliferation and osteogenic differentiation. This approach offers a novel strategy for bone defect repair through the synergistic effects of physical barriers, chemical induction, and physical stimulation.[97] Risangud developed a self-healing hydrogel fabricated through SLA technology, composed of CMCS and oxidized dextran (ODex). By tuning the concentration of CMCS and the oxidation level

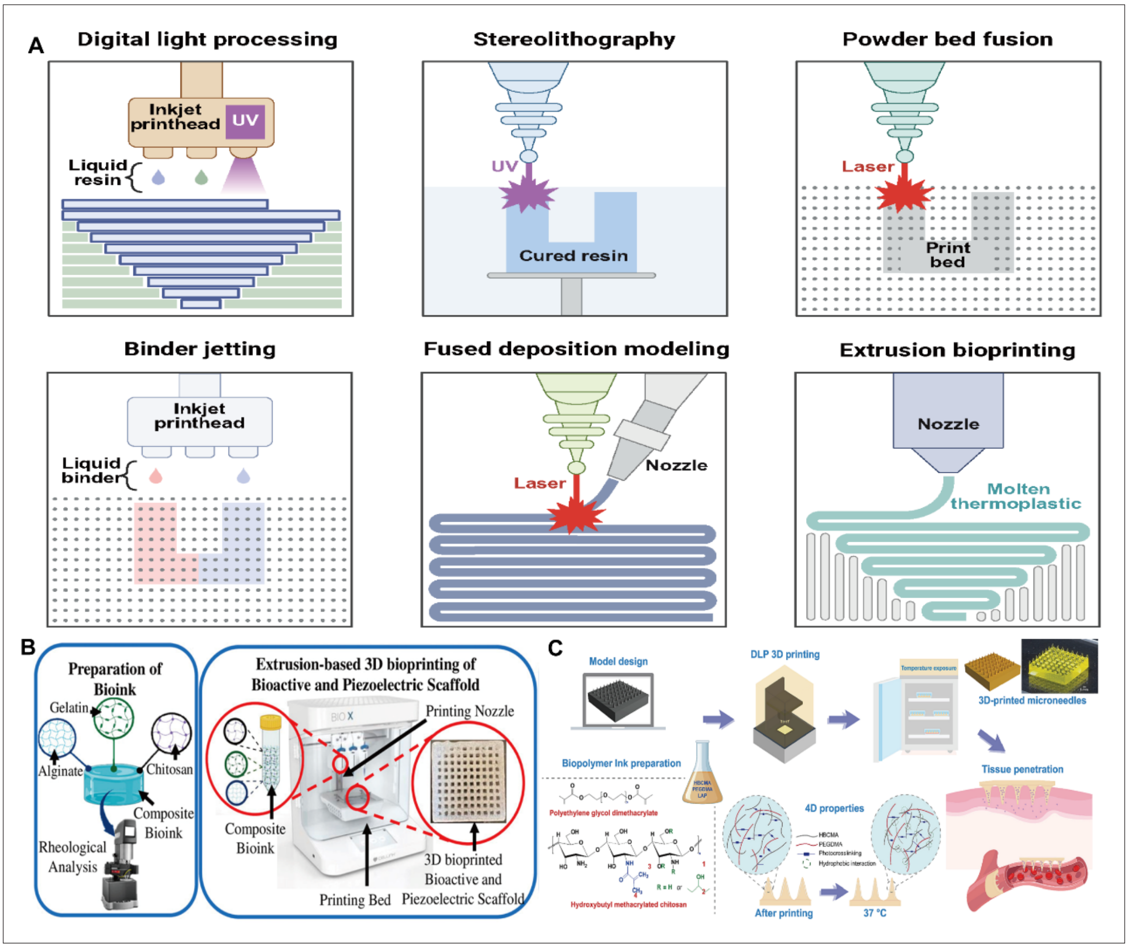

Figure 6. 3D Printing technology based on chitosan hydrogels. (A) Schematic diagram of the most common 3D printing techniques (created in https://BioRender.com). (B) Extrusion-based 3D printing technology. Reprinted with permission from Ref. [94]. Copyright © 2025, Mary Ann Liebert, Inc. (C) Fabrication of hydroxybutyl methacrylate chitosan microneedles using digital light processing (DLP) 3D printing. Reprinted with permission from Ref. [95]. Copyright © 2025, Elsevier. Abbreviations: HBCMA: Hydroxybutyl methacrylated chitosan; LAP: Lithium phenyl-2,4,6-trimethylbenzoylphosphinate; PEGDMA: Polyethylene glycol dimethacrylate; UV: Ultraviolet.

of ODex, the mechanical characteristics and biological performance of the hydrogel could be effectively tailored.[98]

However, SLA processing requires low-viscosity bioinks, which limits cell encapsulation and filler incorporation. Light scattering, shallow penetration depth, and photoinitiator-induced cytotoxicity often compromise curing uniformity and biocompatibility.[99] Moreover, methacrylation may alter chitosan degradation and reduce mechanical strength, making SLA less suitable for load-bearing or high-cell-density constructs.[76] Future efforts should focus on visible-light or enzyme-initiated crosslinking, dual-network and nanocomposite formulations, and hybrid SLA–extrusion systems to enhance mechanical robustness and biological functionality. SLA's precision and reproducibility make it particularly advantageous for fabricating cartilage-layer architectures and microstructured osteochondral interfaces where fine resolution outweighs load-bearing requirements.

7.3. Fused deposition modeling

With FDM technology, thermoplastic materials are heated to a molten state and then deposited layer by layer through a nozzle onto the build platform, ultimately forming a 3D scaffold. FDM offers a cost-effective, scalable, and structurally stable platform for fabricating osteochondral scaffolds. When integrated with chitosan-based composites such as polylactic acid (PLA), PCL, or surface-modified chitosan filaments, FDM enables precise construction of load-bearing subchondral frameworks with tunable porosity and mechanical strength. Its high shape fidelity and design flexibility make it particularly suitable for bone-side scaffolds requiring mechanical support and anatomical conformity. In one study, chitosan was incorporated to reinforce the mechanical performance of PLA scaffolds fabricated via FDM technology.[100] Findings revealed that the mechanical strength of PLA/chitosan composites is strongly influenced by the chitosan loading content and packing density. Because FDM is restricted to thermoplastic materials, chitosan is often employed as a surface-modification agent to enhance the biocompatibility and bioactivity of printed polymers. One study utilized chitosan to enhance the mechanical properties of PLA scaffolds, with the material printed using an FDM setup.[101] Singh *et al.*[100] fabricated PLA/PEG 3D-printed scaffolds coated with chitosan–PEG nanofibers loaded with vancomycin and IGF-1, achieving mechanical support, antibacterial properties, and enhanced osteointegration for bone repair. Some researchers directly coated scaffolds with chitosan solution. For instance, Ye and colleagues[102] fabricated 3D printed poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) scaffolds and subsequently coated them with chitosan. Compared to uncoated PHBV constructs, the chitosan-coated scaffolds significantly enhanced the adhesion and proliferation of BMSCs and promoted osteogenic differentiation by upregulating osteogenesis-related gene expression.[102] With ongoing innovations in materials science and manufacturing precision, FDM demonstrates strong potential for osteochondral tissue engineering applications. Continued optimization of the mechanical strength, structural accuracy, and biodegradability of chitosan-based composite scaffolds is expected to further facilitate their translation into clinical therapies.

However, the non-meltable and hydrophilic nature of native chitosan limits its direct processability and compatibility with hydrophobic thermoplastics. High chitosan content can reduce filament printability and mechanical integrity, while poor interfacial adhesion between the printed polymer framework and subsequent hydrogel layers impairs overall integration.[103,104] Consequently, FDM is best applied as a supportive structural framework, combined with extrusion or hydrogel-based deposition to create hybrid osteochondral constructs that couple strength with bioactivity.[105] Future research should focus on surface grafting and compatibilizer modification to improve polymer–biopolymer bonding, gradient co-printing to achieve continuous transitions across bone–cartilage regions, and bioactive filler incorporation to enhance osteoconductivity. Integration of multi-material and hybrid FDM–bioprinting systems will further enable the fabrication of patient-specific, mechanically functional, and biologically responsive osteochondral scaffolds.

7.4. Digital light processing

Digital light processing (DLP) technology utilizes a digital light source (such as ultraviolet light) to project light onto the surface of photosensitive resin, causing it to cure layer by layer in the illuminated areas and rapidly form intricate 3D structures.[106] DLP technology is capable of achieving submicron-level printing precision, making it suitable for manufacturing complex osteochondral scaffolds, particularly those requiring intricate pore structures and smooth surface finishes. However, DLP technology imposes stringent material requirements. In osteochondral scaffold design, chitosan often requires integration with complementary materials to improve its mechanical performance and augment its biological functionality.[107] A study utilized PEGDA as the base material, combined with chitosan, to fabricate a multi-channel nerve-guiding conduit via DLP printing. This approach achieved structural guidance and bio-induced synergistic repair of peripheral nerve injuries.[60] Furthermore, chitosan microneedles produced by DLP technology have been employed for the controlled administration of small-molecule pharmaceuticals, exhibiting outstanding soft tissue penetration (Figure 6C).

Additionally, DLP requires low-viscosity bioinks, limiting cell density and the inclusion of reinforcing fillers.[108] Light scattering and restricted penetration depth can result in incomplete curing in thick constructs, while cytotoxicity of photoinitiators and potential photothermal stress remain challenges for biocompatibility.[99] Moreover, the mechanical strength of DLP-printed chitosan hydrogels is typically insufficient for load-bearing applications, restricting their use to cartilage or interface regions rather than the subchondral bone zone. In practice, DLP excels in engineering high-fidelity cartilage-like zones, zonal gradient architectures, and bioactive coatings that demand precision rather than bulk strength. Its combination with extrusion-based deposition or FDM frameworks enables multi-material osteochondral constructs with both structural integrity and biological functionality. Future development will focus on visible-light and enzyme-initiated photopolymerization to enhance cytocompatibility, nanocomposite reinforcement to improve modulus, and dual-network hydrogel systems to achieve balanced strength and elasticity. Integration of multi-photon and grayscale DLP techniques will further expand its potential for biomimetic gradient and multilayer cartilage–bone interfaces.

8. Conclusions and future perspectives

Chitosan-based 3D-printed scaffolds exhibit significant potential in osteochondral repair. These scaffolds, fabricated using technologies like FDM, low-temperature deposition, and photopolymerization SLA, offer controllable porous structures that enhance cell migration, adhesion, and nutrient delivery. Compared with other printing methods, EBP has the broadest adaptability to ink viscosity and is particularly suitable for chitosan-based inks. The extrusion method enables stable deposition of high-viscosity polymer hydrogel precursor materials, even those with high cell density, a characteristic demonstrated in previous studies.[112,113] Several studies have identified high viscosity, shear thinning, and rapid shape recovery as key rheological characteristics that facilitate high-conformal printing with chitosan-based inks.[2,114,115] Correspondingly, the extrusion method can satisfy the combined requirements of extrudability, continuity, interlayer support, and conformability within a broad process window. In recent years, researchers have further expanded the printability and mechanical limits of chitosan through strategies such as freeze-dried/rehydrated inks composed of chitosan nanofibers/hyaluronic acid blends or direct printing of high-strength chitosan hydrogels. These systems have achieved high printing resolution and excellent cell compatibility on extrusion platforms, highlighting the extrusion method's adaptability advantages for chitosan.[116] The integration of chitosan with synthetic polymers and bio-ceramics improves mechanical strength, enabling the scaffolds to withstand joint loading. In terms of bioactivity, chitosan-based composite scaffolds support osteoblast and chondrocyte growth, with modifications such as methacrylation and composite bioinks enhancing printability and cellular compatibility. Incorporating

Table 2. Summary of limitations, disadvantages, and applications of different 3D printing techniques combined with chitosan-based biomaterials for osteochondral repair

Printing Method	Limitations	Disadvantages	Future directions	Application scenarios	Ref.
EBP	Nozzle clogging; limited stability; low resolution	Difficulty balancing elasticity and stiffness; weak layer adhesion under cyclic load	Dual crosslinking; coaxial printing; microgel reinforcement; nanoparticle fillers	Cell-laden bioinks; bilayer and gradient structure construction	84,92,93
Extrusion + photocuring	Cytotoxic; light scattering; poor mechanical properties	Nonuniform curing in thick constructs; limited wear/ lubrication; poor fatigue strength	Visible-light, low-tox initiators (e.g., LAP); two-step curing; nanoparticle reinforcement	Moderate-resolution bioprinting; multilayer constructs; fast gelation; tunable stiffness	109-111
DLP/SLA	Poor cell loading; phototoxicity; incomplete deep curing; complex setup	Poor wear resistance; poor fatigue resistance	Visible-light initiators; filler reinforcement; gradient structures via DLP	High-precision fabrication; mimicking native cartilage	76,99,108
FDM	Non-meltable; poor adhesion; low mechanical strength	Poor interface bonding	Surface modification; coupling agents; gradient co-printing	Cost-efficient fabrication; subchondral bone scaffolds; mechanical support	103-105

Abbreviations: DLP: Digital light processing; EBP: Extrusion-based bioprinting; FDM: Fused deposition modeling; LAP: Lithium phenyl-2,4,6-trimethylbenzoylphosphinate; SLA: Stereolithography.

bioactive factors such as bone morphogenetic protein 2 and TGF-β facilitates controlled release, promoting osteogenesis and chondrogenesis at specific defect sites. In cell delivery and tissue integration, 3D bioprinting enables the precise placement of MSCs, chondrocytes, or osteoblasts within chitosan hydrogels. Dual- or triple-nozzle systems allow for the creation of biphasic or triphasic scaffolds that mimic the osteochondral structure. Combined with exosome-based acellular therapies, these approaches improve cell viability, maintain stem cell potency, and modulate the immune microenvironment, offering new strategies for tissue regeneration.

Although significant advancements have been achieved in the development of chitosan-based 3D printed scaffolds, numerous obstacles still hinder their translation from experimental research to routine clinical use, highlighting key areas for future investigation. Future research should focus on developing novel chitosan derivatives and composite materials. For example, chemical modifications such as phosphorylation, carboxymethylation, and quaternization can not only improve solubility but also introduce new biological functions. Additionally, exploring the incorporation of nanomaterials, novel synthetic hydrogels, or high-toughness polymers can help achieve mechanical properties that match native tissues, or even feature gradient variations, while maintaining biological activity. The current resolution, speed, and multi-material capabilities of 3D printing technologies still leave room for improvement. Future printing technologies with higher precision will enable the creation of finer microstructures that better replicate the nanoscale topology of the ECM. 4D bioprinting, introducing time as the fourth dimension, will allow printed scaffolds to undergo pre-programmed shape or functional transformations in response to external stimuli after implantation, opening new pathways for minimally invasive surgery and dynamic tissue remodeling. Furthermore, *in situ* 3D printing technology, which allows direct printing at the defect site, will significantly reduce surgical time and improve the fitting of implants to irregular defects, making it an attractive frontier.

Future scaffolds should not only provide physical support but also act as intelligent systems that dynamically respond to and guide the regeneration process. This includes: precise delivery of bioactive factors, requiring the development of more advanced controlled-release systems capable of sequential, spatiotemporal delivery of multiple growth factors to accurately simulate the natural healing cascade; immune modulation, through scaffolds with active immunoregulatory functions—such as integrating anti-inflammatory drugs or using chitosan and its composites to induce macrophage polarization toward the M2 anti-inflammatory phenotype—to create a favorable immune microenvironment for regeneration. It is also necessary to optimize bioink formulations, printing parameters, and crosslinking methods to maximize cell viability. For large-scale bone regeneration, promoting rapid vascularization within the scaffold is a key factor for success. Future designs may incorporate specific angiogenic factors or pre-constructed microchannel networks. Finally, establishing standardized quality control and safety evaluation systems, followed by large-scale, long-term preclinical and clinical studies, is an essential step toward transitioning chitosan-based 3D printed scaffolds from the laboratory to the market.

In conclusion, chitosan-based 3D printed scaffolds have demonstrated great potential and vitality in the field of osteochondral repair. Through interdisciplinary collaboration—integrating the latest advancements in materials science, bioengineering, mechanical manufacturing, and clinical medicine—and by continuously overcoming existing challenges, we are optimistic about achieving truly personalized and functional osteochondral regeneration in the near future, bringing hope to countless patients.

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Conflict of interest

The author declares no conflict of interest, financial or otherwise.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

References

1. Lu J, Gao Y, Cao C, *et al*. 3D bioprinted scaffolds for osteochondral regeneration: advancements and applications. *Mater Today Bio*. 2025;32:101834. doi: 10.1016/j.mtbio.2025.101834
2. Taghizadeh M, Taghizadeh A, Yazdi MK, *et al*. Chitosan-based inks for 3D printing and bioprinting. *Green Chem*. 2022; 24(1), 62-101. doi: 10.1039/D1GC01799C
3. Lin X, Zhang Y, Li J, *et al*. Biomimetic multizonal scaffolds for the reconstruction of zonal articular cartilage in chondral and osteochondral defects. *Bioact Mater*. 2025;43: 510-549. doi: 10.1016/j.bioactmat.2024.10.001
4. Zhang G, He S, He X, *et al*. Local Recruitment of Autologous Stem Cells via a Targeting Microgel for Precise Cartilage Repair without Surgery. *Adv Mater*. 2025;37(33):e2505544. doi: 10.1002/adma.202505544
5. Zhang B, Zhang M, Jiang C, Yan W, Pan Y, Meng F. Engineered polysaccharide scaffolds for cartilage regeneration: Mechanisms, functionalization, and clinical prospects. *Colloids Surf B Biointerfaces*. 2025;257:115134. doi: 10.1016/j.colsurfb.2025.115134
6. Kunze KN, Persaud S, Briano J, *et al*. Outcomes of Revision Cartilage Restoration Surgery for Failed Primary Treatment of Chondral or Osteochondral Defects of the Knee: A Systematic Review. *Am J Sports Med*. 2025;53(6): 1515-1523. doi: 10.1177/03635465241260271
7. Nguyen MN, Vu BT, Truong DM, *et al*. Fabrication of 3-Dimensional-Printed Bilayered Scaffold Carboxymethyl Chitosan/Oxidized Xanthan Gum, Biphasic Calcium Phosphate for Osteochondral Regeneration. *Biomater Res*. 2025;29:0186. doi: 10.34133/bmr.0186
8. Wang X, Wu S, Li R, *et al*. ROS-Activated Nanohydrogel Scaffolds with Multi-Factors Controlled Release for Targeted Dual-Lineage Repair of Osteochondral Defects. *Adv Sci (Weinh)*. 2025;12(20):e2412410. doi: 10.1002/advs.202412410
9. Koh RH, Kim J, Kim JU, *et al*. Bioceramic-mediated chondrocyte hypertrophy promotes calcified cartilage formation for rabbit osteochondral defect repair. *Bioact Mater*. 2024;40:306-317. doi: 10.1016/j.bioactmat.2024.06.018
10. Wu Z, Yao H, Sun H, *et al*. Enhanced hyaline cartilage formation and continuous osteochondral regeneration via 3D-Printed heterogeneous hydrogel with multi-crosslinking inks. *Mater Today Bio*. 2024;26:101080. doi: 10.1016/j.mtbio.2024.101080
11. Wu H, Chen C, Li J, *et al*. Engineered Magneto-Piezoelectric Nanoparticles-Enhanced Scaffolds Disrupt Biofilms and Activate Oxidative Phosphorylation in Icam1(+) Macrophages for Infectious Bone Defect Regeneration. *ACS Nano*. 2024;18(52):35575-35594. doi: 10.1021/acsnano.4c13562
12. Wang Y, Zhou X, Jiang J, *et al*. Carboxymethyl chitosan-enhanced multi-level microstructured composite hydrogel scaffolds for bone defect repair. *Carbohydr Polym*. 2025;348(Pt B):122847. doi: 10.1016/j.carbpol.2024.122847
13. Wei P, Zhou J, Xiong S, *et al*. Chestnut-Inspired Hollow Hydroxyapatite 3D Printing Scaffolds Accelerate Bone Regeneration by Recruiting Calcium Ions and Regulating Inflammation. *ACS Appl Mater Interfaces*. 2024;16(8):9768-9786. doi: 10.1021/acsami.3c17087
14. Ngau SM, Cheah KH, Wong VL, Khiew PS, Lim SS. 3D-printed poly(ethylene) glycol diacrylate (PEGDA)-chitosan-nanohydroxyapatite scaffolds: Structural characterization and cellular response. *Int J Biol Macromol*. 2025;296:139652. doi: 10.1016/j.ijbiomac.2025.139652
15. Hu X, Man Y, Li W, *et al*. 3D Bio-Printing of CS/Gel/HA/Gr Hybrid Osteochondral Scaffolds. *Polymers (Basel)*. 2019;11(10). doi: 10.3390/polym11101601
16. Xu J, Su Y, Yang J, *et al*. A 3D bioprinted gelatin/quaternized chitosan/decellularized extracellular matrix based hybrid bionic scaffold with multifunctionality for infected full-

thickness skin wound healing. *Int J Biol Macromol*. 2025;309(Pt 1):142816.
doi: 10.1016/j.ijbiomac.2025.142816

17. Zhang W, Shi K, Yang J, *et al*. 3D printing of recombinant collagen/chitosan methacrylate/nanoclay hydrogels loaded with Kartogenin nanoparticles for cartilage regeneration. *Regen Biomater*. 2024;11:rbae097.
doi: 10.1093/rb/rbae097
18. Corrado F, Di Maio L, Palmero P, *et al*. Vat photo-polymerization 3D printing of gradient scaffolds for osteochondral tissue regeneration. *Acta Biomater*. 2025;200:67-86.
doi: 10.1016/j.actbio.2025.05.042
19. Jacob G, Shimomura K, Nakamura N. Osteochondral Injury, Management and Tissue Engineering Approaches. *Front Cell Dev Biol*. 2020;8:580868.
doi: 10.3389/fcell.2020.580868
20. Wu H, Wang X, Wang G, *et al*. Advancing Scaffold-Assisted Modality for In Situ Osteochondral Regeneration: A Shift From Biodegradable to Bioadaptable. *Adv Mater*. 2024;36(47):e2407040.
doi: 10.1002/adma.202407040
21. Kwon H, Brown WE, Lee CA, *et al*. Surgical and tissue engineering strategies for articular cartilage and meniscus repair. *Nat Rev Rheumatol*. 2019;15(9):550-570.
doi: 10.1038/s41584-019-0255-1
22. Gao F, Xu Z, Liang Q, *et al*. Direct 3D Printing of High Strength Biohybrid Gradient Hydrogel Scaffolds for Efficient Repair of Osteochondral Defect. *Adv Funct Mater*. 2018;28(13):1706644.
doi: 10.1002/adfm.201706644
23. Gu Y, Zou Y, Huang Y, *et al*. 3D-printed biomimetic scaffolds with precisely controlled and tunable structures guide cell migration and promote regeneration of osteochondral defect. *Biofabrication*. 2023;16(1):015003.
doi: 10.1088/1758-5090/ad0071
24. Qu H, Gao C, Liu K, *et al*. Gradient matters via filament diameter-adjustable 3D printing. *Nat Commun*. 2024;15(1):2930.
doi: 10.1038/s41467-024-47360-y
25. Li CJ, Park JH, Jin GS, *et al*. Strontium/Silicon/Calcium-Releasing Hierarchically Structured 3D-Printed Scaffolds Accelerate Osteochondral Defect Repair. *Adv Healthc Mater*. 2024;13(20):e2400154.
doi: 10.1002/adhm.202400154
26. Li Q, Yu H, Zhao F, *et al*. 3D Printing of Microenvironment-Specific Bioinspired and Exosome-Reinforced Hydrogel Scaffolds for Efficient Cartilage and Subchondral Bone Regeneration. *Adv Sci (Weinh)*. 2023;10(26):e2303650.
doi: 10.1002/advs.202303650
27. Li C, Wang K, Li T, *et al*. Patient-specific Scaffolds with a Biomimetic Gradient Environment for Articular Cartilage-Subchondral Bone Regeneration. *ACS Appl Bio Mater*. 2020;3(8):4820-4831.
doi: 10.1021/acsabm.0c00334
28. Jahani A, Nourbakhsh MS, Moradi A, Mohammadi M, Tayebi L. Incorporating insulin into alginate-chitosan 3D-printed scaffolds: A comprehensive study on structure, mechanics, and biocompatibility for cartilage tissue engineering. *Carbohydr Polym Technol Appl*. 2025;9:100696.
doi: 10.1016/j.carpta.2025.100696
29. Kang Y, Xu J, Meng L, *et al*. 3D bioprinting of dECM/Gel/QCS/nHAp hybrid scaffolds laden with mesenchymal stem cell-derived exosomes to improve angiogenesis and osteogenesis. *Biofabrication*. 2023;15(2):024103.
doi: 10.1088/1758-5090/acb6b8
30. Wang W, Zheng L, Liu W, *et al*. Enhanced efficacy of 3D architectural glass–ceramic scaffolds in vertical bone augmentation compared with conventional bone-block grafts of different origins. *Mater Des*. 2025;253,113989.
doi: 10.1016/j.matdes.2025.113989
31. Sun Q, Qiu T, Liu X, Wei Q. Cellular Spatial Sensing Determines Cell Mechanotransduction Activity on the Aligned Nanofibers. *Small*. 2025;21(13):e2410351.
doi: 10.1002/smll.202410351
32. Cho S, Lee KS, Lee K, *et al*. Surface Crystal and Degradability of Shape Memory Scaffold Essentialize Osteochondral Regeneration. *Small*. 2024;20(40):e2401989.
doi: 10.1002/smll.202401989
33. Tang W, Pan P, Chen T, *et al*. 3D chitosan scaffolds loaded with ZnO nanoparticles for bone tissue engineering. *Colloids Surf B Biointerfaces*. 2025;245:114199.
doi: 10.1016/j.colsurfb.2024.114199
34. Zhao W, Chen H, Zhang Y, *et al*. Adaptive multi-degree-of-freedom in situ bioprinting robot for hair-follicle-inclusive skin repair: A preliminary study conducted in mice. *Bioeng Transl Med*. 2022;7(3):e10303.
doi: 10.1002/btm2.10303
35. Lipskas J, Deep K, Yao W. Robotic-Assisted 3D Bio-printing for Repairing Bone and Cartilage Defects through a Minimally Invasive Approach. *Sci Rep*. 2019;9(1):3746.
doi: 10.1038/s41598-019-38972-2
36. Shariatinia Z. Carboxymethyl chitosan: Properties and biomedical applications. *Int J Biol Macromol*. 2018;120(Pt B):1406-1419.
doi: 10.1016/j.ijbiomac.2018.09.131
37. Liu Y, Yuan K, Lin Y, *et al*. Directional Freeze-Casting Cryogel Loaded with Quaternized Chitosan Modified Gallium Metal-Organic Frameworks to Capture and Eradicate the Resistant Bacteria for Guided Regeneration in Infected Bone Defects. *Adv Mater*. 2025;37(9):e2414437.
doi: 10.1002/adma.202414437
38. Chen YR, Yan X, Yuan FZ, *et al*. Kartogenin-Conjugated Double-Network Hydrogel Combined with Stem Cell

Transplantation and Tracing for Cartilage Repair. *Adv Sci (Weinh)*. 2022;9(35):e2105571.
doi: 10.1002/advs.202105571

39. Islam MM, Shahruzzaman M, Biswas S, Nurus Sakib M, Rashid TU. Chitosan based bioactive materials in tissue engineering applications-A review. *Bioact Mater*. 2020;5(1):164-183.
doi: 10.1016/j.bioactmat.2020.01.012

40. Furuike T, Komoto D, Hashimoto H, Tamura H. Preparation of chitosan hydrogel and its solubility in organic acids. *Int J Biol Macromol*. 2017;104(Pt B):1620-1625.
doi: 10.1016/j.ijbiomac.2017.02.099

41. Jirofti N, Hashemi M, Moradi A, Kalalinia F. Fabrication and characterization of 3D printing biocompatible crocin-loaded chitosan/collagen/hydroxyapatite-based scaffolds for bone tissue engineering applications. *Int J Biol Macromol*. 2023;252:126279.
doi: 10.1016/j.ijbiomac.2023.126279

42. Kumi M, Wang T, Ejeromedoghene O, Wang J, Li P, Huang W. Exploring the Potentials of Chitin and Chitosan-Based Bioinks for 3D-Printing of Flexible Electronics: The Future of Sustainable Bioelectronics. *Small Methods*. 2024;8(9):e2301341.
doi: 10.1002/smtd.202301341

43. Jiang T, Yang Y, Lin Z, Hong Y, Luo Z. Modified Polysaccharides: Potential Biomaterials for Bioprinting. *J Funct Biomater*. 2025;16(9):338.
doi: 10.3390/jfb16090338

44. Qin S, Wang W, Chen L, *et al*. 3D printed osteochondral lineage-specific biphasic scaffolds for functional repair of full-thickness articular cartilage defects in weight-bearing area. *Biofabrication*. 2025;17(3):035025.
doi: 10.1088/1758-5090/ade8a9

45. Liu K, Zhang Y, Huang L, *et al*. Enhanced printability of high-viscosity chitosan/acrylamide inks via aluminum ions coordination for precision 3D bioprinting of scaffolds. *Carbohydr Polym*. 2025;355:123359.
doi: 10.1016/j.carbpol.2025.123359

46. Chang HK, Yang DH, Ha MY, *et al*. 3D printing of cell-laden visible light curable glycol chitosan bioink for bone tissue engineering. *Carbohydr Polym*. 2022;287:119328.
doi: 10.1016/j.carbpol.2022.119328

47. Lin M, Stehle Y, Chen L, *et al*. A 3D-printed chitosan-based pH-responsive dual functional scaffold for osteomyelitis: synergistic antibacterial and osteogenic treatment. *Carbohydr Polym*. 2025;366:123866.
doi: 10.1016/j.carbpol.2025.123866

48. Liu X, Wang Y, Zhang W, *et al*. Caffeic acid and adenine modified chitosan dual-network hydrogel with antioxidant and pro-proliferative properties for diabetic wound healing. *Carbohydr Polym*. 2025;369:124290.
doi: 10.1016/j.carbpol.2025.124290

49. Wang X, Zhang J, Shan J, *et al*. Polypyrrole-Modified Triple-Responsive Hydrogel Dressing Based on Bacterial Cellulose and Quaternary Ammonium Chitosan and Its Synergistic Antibacterial Mechanism. *ACS Appl Mater Interfaces*. 2025;17(36):50417-50431.
doi: 10.1021/acsami.5c12061

50. Liu Y, Zhou C, Zhang X, Liu W. Chirality Hydroxyapatite Gradient Scaffold Drives Osteochondral Regeneration via YAP/TAZ-Mediated Mechanotransduction. *Adv Healthc Mater*. 2025;14(22):e2501668.
doi: 10.1002/adhm.202501668

51. Pitrolino KA, Felfel RM, Pellizzeri LM, *et al*. Development and in vitro assessment of a bi-layered chitosan-nano-hydroxyapatite osteochondral scaffold. *Carbohydr Polym*. 2022;282:119126.
doi: 10.1016/j.carbpol.2022.119126

52. Cui R, Lu Q, Teng Y, Li K, Li N. Chitosan Promoted the Corneal Epithelial Wound Healing via Activation of ERK Pathway. *Curr Eye Res*. 2017;42(1):21-27.
doi: 10.3109/02713683.2016.1145235

53. Hoemann CD, Rodríguez González J, Guzmán-Morales J, Chen G, Jalali Dil E, Favis BD. Chitosan coatings with distinct innate immune bioactivities differentially stimulate angiogenesis, osteogenesis and chondrogenesis in poly-caprolactone scaffolds with controlled interconnecting pore size. *Bioact Mater*. 2022;10:430-442.
doi: 10.1016/j.bioactmat.2021.09.012

54. Olza S, Hadj Bouzidi NM, Rubatat L, *et al*. Mineralized chitin nanocrystals enhance osteoinductive ability of chitosan 3D porous biohybrid scaffolds for bone tissue regeneration. *Carbohydr Polym*. 2025;366:123911.
doi: 10.1016/j.carbpol.2025.123911

55. Tu C, Gao X, Zheng H, *et al*. Innovative injectable, self-healing, exosome cross-linked biomimetic hydrogel for cartilage regeneration. *J Control Release*. 2025;381:113608.
doi: 10.1016/j.jconrel.2025.113608

56. Tikakosol P, Topham PD, Derry MJ, *et al*. Kartogenin-encapsulated self-healing injectable hydrogel based on hyaluronic acid and chitosan derivative for use as viscosupplementation in knee osteoarthritis. *Int J Biol Macromol*. 2025;328(Pt 1):147304.
doi: 10.1016/j.ijbiomac.2025.147304

57. Li P, Fu L, Liao Z, *et al*. Chitosan hydrogel/3D-printed poly(ε-caprolactone) hybrid scaffold containing synovial mesenchymal stem cells for cartilage regeneration based on tetrahedral framework nucleic acid recruitment. *Biomaterials*. 2021;278:121131.
doi: 10.1016/j.biomaterials.2021.121131

58. Xu J, Ji J, Jiao J, *et al*. 3D Printing for Bone-Cartilage Interface Regeneration. *Front Bioeng Biotechnol*. 2022;10:828921.
doi: 10.3389/fbioe.2022.828921

59. Fan D, Liu Y, Wang Y, *et al*. 3D printing of bone and cartilage with polymer materials. *Front Pharmacol*. 2022;13:1044726.

doi: 10.3389/fphar.2022.1044726

60. Pourfaraj A, Najmoddin N, Behzadnasab M, Pedram MS, Pezeshki-Modaress M. Rapid continuous 3D printed multi-channel poly(ethylene glycol) diacrylate/chitosan nerve guidance conduit: In vivo study. *Int J Biol Macromol*. 2025;321(Pt 4):146491.
doi: 10.1016/j.ijbiomac.2025.146491

61. Wang Z, Sun Y, Li C. Advances in 3D printing technology for preparing bone tissue engineering scaffolds from biodegradable materials. *Front Bioeng Biotechnol*. 2024;12:1483547.
doi: 10.3389/fbioe.2024.1483547

62. Liu G, Wei X, Zhai Y, *et al*. 3D printed osteochondral scaffolds: design strategies, present applications and future perspectives. *Front Bioeng Biotechnol*. 2024;12: 1339916.
doi: 10.3389/fbioe.2024.1339916

63. Novotná R, Franková J. Materials Suitable for Osteochondral Regeneration. *ACS Omega*. 2024;9(28):30097-30108.
doi: 10.1021/acsomega.4c04789

64. Li S, Liu J, Liu S, Jiao W, Wang X. Chitosan oligosaccharides packaged into rat adipose mesenchymal stem cells-derived extracellular vesicles facilitating cartilage injury repair and alleviating osteoarthritis. *J Nanobiotechnology*. 2021;19(1):343.
doi: 10.1186/s12951-021-01086-x

65. Chen Y, Mehmood K, Chang YF, Tang Z, Li Y, Zhang H. The molecular mechanisms of glycosaminoglycan biosynthesis regulating chondrogenesis and endochondral ossification. *Life Sci*. 2023;335:122243.
doi: 10.1016/j.lfs.2023.122243

66. Mochizuki M, Yamagata N, Philp D, *et al*. Integrin-dependent cell behavior on ECM peptide-conjugated chitosan membranes. *Biopolymers*. 2007;88(2):122-30.
doi: 10.1002/bip.20684

67. Yeh HY, Liu BH, Sieber M, Hsu SH. Substrate-dependent gene regulation of self-assembled human MSC spheroids on chitosan membranes. *BMC Genomics*. 2014;15(1):10.
doi: 10.1186/1471-2164-15-10

68. Xu B, Ye J, Song S, *et al*. Inherently bioactive iron-chelating Poly (N-acryloyl 2-glycine)/chitosan hydrogel scaffolds orchestrating dual hypoxic-immune microenvironment for functional meniscus regeneration. *Bioact Mater*. 2025;54:492-508.
doi: 10.1016/j.bioactmat.2025.08.028

69. Liu H, Liu S, Liao L, Liao H, Pan W, Hu J. Dual stimulus-responsive dECM cryogel sequentially regulate bone microenvironment for one-step surgical therapy of chronic osteomyelitis. *Mater Today Bio*. 2025;34:102243.
doi: 10.1016/j.mtbio.2025.102243

70. He S, Lin B, Zhang C, *et al*. Injectable chitosan-based thermosensitive hydrogel loaded with adipose-derived mesenchymal stem cells promotes pressure ulcer healing. *Colloids Surf B Biointerfaces*. 2025;256(Pt 2):115089.
doi: 10.1016/j.colsurfb.2025.115089

71. Hia EM, Park J, Suh IW, Park CH. Synergistic effects of modified zinc oxide nanoparticle in a hybrid chitosan-gelatin hydrogel for bone regeneration. *Int J Biol Macromol*. 2025;315(Pt 2):144490.
doi: 10.1016/j.ijbiomac.2025.144490

72. Mokhtari H, Bahari M, Yeganeh F. Chitosan-based Biomaterials in Regenerative Medicine: Optimizing Mesenchymal Stem Cell Viability and Function. *Stem Cell Rev Rep*. 2025;21(7):2010-2030.
doi: 10.1007/s12015-025-10901-z

73. Liu XL, Zhang CJ, Shi JJ, *et al*. Nacre-mimetic cerium-doped nano-hydroxyapatite/chitosan layered composite scaffolds regulate bone regeneration via OPG/RANKL signaling pathway. *J Nanobiotechnology*. 2023;21(1):259.
doi: 10.1186/s12951-023-01988-y

74. Pahlevanzadeh F, Emadi R, Kharaziha M, *et al*. Amorphous magnesium phosphate-graphene oxide nano particles laden 3D-printed chitosan scaffolds with enhanced osteogenic potential and antibacterial properties. *Biomater Adv*. 2024;158:213760.
doi: 10.1016/j.bioadv.2024.213760

75. Duan X, Hu K, Wang J, *et al*. Core-shell engineered Col/Cs@ ECM microspheres for macrophage-targeted intracellular drug release in RA therapy. *Bioact Mater*. 2025;54:715-729.
doi: 10.1016/j.bioactmat.2025.08.043

76. Klak M, Kosowska K, Czajka M, *et al*. The Impact of the Methacrylation Process on the Usefulness of Chitosan as a Biomaterial Component for 3D Printing. *J Funct Biomater*. 2024;15(9):251.
doi: 10.3390/jfb15090251

77. Chen XD, Zhang XY, Zhu HQ, Lu HH, Wang M. Three-Dimensional Printing of Hydrogel Blend Tissue Engineering Scaffolds with In Situ Delivery of Anticancer Drug for Treating Melanoma Resection-Induced Tissue Defects. *J Funct Biomater*. 2024;15(12).
doi: 10.3390/jfb15120381

78. Ding Y, Zhu J, Guan X, *et al*. Photocrosslinkable hydrogel of kartogenin functionalized chitosan methacrylate preserves chondrogenesis. *Int J Biol Macromol*. 2025;321(Pt 3):146378.
doi: 10.1016/j.ijbiomac.2025.146378

79. Liu Y, Du L, Zhang H, *et al*. Bioprinted biomimetic hydrogel matrices guiding stem cell aggregates for enhanced chondrogenesis and cartilage regeneration. *J Mater Chem B*. 2024;12(22):5360-5376.
doi: 10.1039/d4tb00323c

80. Lingait D, Rahagude R, Gaharwar SS, *et al*. A review on versatile applications of biomaterial/polycationic chitosan: An insight into the structure-property relationship. *Int J Biol Macromol*. 2024;257(Pt 2):128676.
doi: 10.1016/j.ijbiomac.2023.128676

81. Tomal W, Ortyl J. Water-Soluble Photoinitiators in Biomedical Applications. *Polymers (Basel)*. 2020;12(5). doi: 10.3390/polym12051073

82. Wang L, Huang P, Huang J, *et al*. Modeling of molecular weight changes of polysaccharides subjected to irradiation. *Carbohydr Polym Technol Appl*. 2025;10(000):100815. doi: 10.1016/j.carpta.2025.100815

83. Torgal S, Subramani G, Manian R. Comprehensive insights into chitosan hydrogels: from crosslinking and characterization to immunomodulation, microbiome interactions and biomedical uses. *Biomass Convers Biorefin*. 2025;(9):15. doi: 10.1007/s13399-024-06350-2

84. Taghizadeh M, Taghizadeh A, Yazdi MK, *et al*. Chitosan-based inks for 3D printing and bioprinting (vol 24, pg 62, 2022). *Green Chem*. 2025;(18):27. doi: 10.1039/d5gc90073e

85. Afra S, Samadi A, Asadi P, *et al*. Chitosan crosslinkers and their functionality in 3D bioprinting to produce chitosan-based bioinks. *Inorg Chem Commun*. 2024;168(000):12. doi: 10.1016/j.inoche.2024.112842

86. Williams CG, Malik AN, Kim TK, Manson PN, Elisseeff JH. Variable cytocompatibility of six cell lines with photoinitiators used for polymerizing hydrogels and cell encapsulation. *Biomaterials*. 2005;26(11):1211-8. doi: 10.1016/j.biomaterials.2004.04.024

87. Pitrolino K, Felfel R, Roberts G, Scotchford C, Grant D, Sottile V. In vitrodegradation of a chitosan-based osteochondral construct points to a transient effect on cellular viability. *Biomed Mater*. 2024;19(5):055025. doi: 10.1088/1748-605X/ad6547

88. Rahayu DP, Yunus AL, Yunus MY, *et al*. Study the effect of gamma-irradiation on the physical properties of Chitosan powder with the presence of water. *AIP Conf Proc*. 2024;3074(1):5. doi: 10.1063/5.0211274

89. Cowie RM, Macri-Pellizzeri L, Mclaren J, *et al*. Functional performance of a bi-layered chitosan-nano-hydroxyapatite osteochondral scaffold: a pre-clinical in vitro tribological study. *R Soc Open Sci*. 2024;11(1):13. doi: 10.1098/rsos.230431

90. Andreica BI, Cheng X, Marin L. Quaternary ammonium salts of chitosan. A critical overview on the synthesis and properties generated by quaternization. *Eur Polym J*. 2022;(164-):164. doi: 10.1016/j.eurpolymj.2020.110016

91. Jergitsch M, Mateos-Timoneda MA. 3D extrusion bioprinting: rational bioink design and advanced fabrication techniques. *Trends Biotechnol*. 2025;S0167-7799(25)00223-9. doi: 10.1016/j.tibtech.2025.06.008

92. Agarwal T, Chiesa I, Costantini M, *et al*. Chitosan and its derivatives in 3D/4D (bio) printing for tissue engineering and drug delivery applications. *Int J Biol Macromol*. 2023;246:125669. doi: 10.1016/j.ijbiomac.2023.125669

93. Chen XB, Fazel Anvari-Yazdi A, Duan X, *et al*. Biomaterials / bioinks and extrusion bioprinting. *Bioact Mater*. 2023;28:511-536. doi: 10.1016/j.bioactmat.2023.06.006

94. Sonaye SY, Bohara S, Welsh BL, *et al*. Extrusion-Based 3D Bioprinting of Bioactive and Piezoelectric Scaffolds as Potential Therapy for Treating Critical Soft Tissue Wounds. *Adv Wound Care (New Rochelle)*. 2025;14(3):143-158. doi: 10.1089/wound.2024.0073

95. Che QT, Seo JW, Charoensri K, Nguyen MH, Park HJ, Bae H. 4D-printed microneedles from dual-sensitive chitosan for non-transdermal drug delivery. *Int J Biol Macromol*. 2024;261(Pt 2):129638. doi: 10.1016/j.ijbiomac.2024.129638

96. Li W, Wang M, Ma H, Chapa-Villarreal FA, Lobo AO, Zhang YS. Stereolithography apparatus and digital light processing-based 3D bioprinting for tissue fabrication. *iScience*. 2023;26(2):106039. doi: 10.1016/j.isci.2023.106039

97. Zhou X, Zou B, Chen Q, Yang G, Lai Q, Wang X. Construction of bilayer biomimetic periosteum based on SLA-3D printing for bone regeneration. *Colloids Surf B Biointerfaces*. 2025;246:114368. doi: 10.1016/j.colsurfb.2024.114368

98. Risangud N, Lertwimol T, Sitthisang S, Wongvitvichot W, Uppanan P, Tanodekaew S. The preparation of 3D-printed self-healing hydrogels composed of carboxymethyl chitosan and oxidized dextran via stereolithography for biomedical applications. *Int J Biol Macromol*. 2025;292:139251. doi: 10.1016/j.ijbiomac.2024.139251

99. Zhang X, Cheng F, Islam MR, Li H. The fabrication of the chitosan-based bioink for in vitro tissue repair and regeneration: A review. *Int J Biol Macromol*. 2024;257(Pt 2):128504. doi: 10.1016/j.ijbiomac.2023.128504

100. Singh S, GurminderPrakash, ChanderRamakrishna, SeeramLamberti, LucianoPruncu, Catalin, I. 3D printed biodegradable composites: An insight into mechanical properties of PLA/chitosan scaffold. *Polym Test*. 2020;89(1):106722. doi: 10.1016/j.polymertesting.2020.106722

101. Guo W, Peng Z, Ning D, *et al*. Chitosan microporous foam filled 3D printed polylactic acid-pearl macroporous scaffold: Dual-scale porous structure, biological and mechanical properties. *Int J Biol Macromol*. 2025;303:140508. doi: 10.1016/j.ijbiomac.2025.140508

102. Ye X, Li L, Lin Z, *et al*. Integrating 3D-printed PHBV/Calcium sulfate hemihydrate scaffold and chitosan hydrogel for enhanced osteogenic property. *Carbohydr Polym*. 2018;202:106-114.

doi: 10.1016/j.carbpol.2018.08.117

103. Hui I, Pasquier E, Solberg A, Agrenius K, Håkansson J, Chinga-Carrasco G. Biocomposites containing poly(lactic acid) and chitosan for 3D printing - Assessment of mechanical, antibacterial and in vitro biodegradability properties. *J Mech Behav Biomed Mater*. 2023;147:106136. doi: 10.1016/j.jmbbm.2023.106136

104. Bera T, Vincent S, Mohanty S. Mechanical Properties of Polylactic Acid/Chitosan Composites by Fused Deposition Modeling. *J Mater Eng Perform*. 2025;34(16):17106-17120. doi: 10.1007/s11665-024-10506-6

105. Blanzeanu E, Marin M, Verziu MN, *et al*. Chitosan-polylactic acid composites: from seafood waste to advanced functional materials for 3D printing. *Adv Compos Hybrid Mater*. 2025;8(1). doi: 10.1007/s42114-024-01131-x

106. Alparslan C, Bayraktar Ş. Advances in Digital Light Processing (DLP) Bioprinting: A Review of Biomaterials and Its Applications, Innovations, Challenges, and Future Perspectives. *Polymers (Basel)*. 2025;17(9). doi: 10.3390/polym17091287

107. Shen Y, Tang H, Huang X, *et al*. DLP printing photocurable chitosan to build bio-constructs for tissue engineering. *Carbohydr Polym*. 2020;235:115970. doi: 10.1016/j.carbpol.2020.115970

108. Zanon M, Cue-López R, Martínez-Campo E, *et al*. Bioderived dyes-mediated vat photopolymerization 3D printing of chitosan hydrogels for tissue engineering. *Addit Manuf*. 2023;69(c):103553. doi: 10.1016/j.addma.2023.103553

109. He Y, Wang F, Wang X, Zhang J, Wang D, Huang X. A photocurable hybrid chitosan/acrylamide bioink for DLP based 3D bioprinting. *Mater Des*. 2021;202(1):109588. doi: 10.1016/j.matdes.2021.109588

110. García-García A, Pérez-Álvarez L, Ruiz-Rubio L, Larrea-Sebal A, Martin C, Vilas-Vilela JL. Extrusion-Based 3D Printing of Photocrosslinkable Chitosan Inks. *Gels*. 2024;10(2):126. doi: 10.3390/gels10020126

111. Banigo AT, Nauta L, Zoetebier B, Karperien M. Hydrogel-Based Bioinks for Coaxial and Triaxial Bioprinting: A Review of Material Properties, Printing Techniques, and Applications. *Polymers (Basel)*. 2025;17(7):917. doi: 10.3390/polym17070917

112. Mccauley PJ, Fromen CA, Bayles AV. Cell viability in extrusion bioprinting: the impact of process parameters, bioink rheology, and cell mechanics. *Rheol Acta*. 2025; 64(9-10):497-515. doi: 10.1007/s00397-025-01504-z

113. Rossi A, Pescara T, Gambelli AM, *et al*. Biomaterials for extrusion-based bioprinting and biomedical applications. *Front Bioeng Biotechnol*. 2024;12:1393641. doi: 10.3389/fbioe.2024.1393641

114. Rajabi M, McConnell M, Cabral J, Ali MA. Chitosan hydrogels in 3D printing for biomedical applications. *Carbohydr Polym*. 2021;260:117768. doi: 10.1016/j.carbpol.2021.117768

115. Elango J, Zamora-Ledezma C. Rheological, Structural, and Biological Trade-Offs in Bioink Design for 3D Bioprinting. *Gels*. 2025;11(8):659. doi: 10.3390/gels11080659

116. Hirami R, Sakai S. Freeze-dryable inks combining chitosan nanofibers and hyaluronic acid for extrusion bioprinting. *Int J Biol Macromol*. 2025;322(Pt 1):146679. doi: 10.1016/j.ijbiomac.2025.146679