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Review / Mini-Review

Smart Chitosan-based Scaffolds for Cartilage Regeneration: From Biomaterial Design to Clinical Applications

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Abstract

Treatment of cartilage injuries remains a crucial clinical challenge due to the intrinsically limited capacity of this tissue for regeneration. Common treatment approaches are often unable to restore the natural structure and the long-term function of articular cartilage. Therefore, tissue engineering emerges as a promising approach that enhances cartilage regeneration using cell integration, scaffolds, and bioactive molecules. Chitosan stands out among various types of bioactive materials owing to its biocompatibility, biodegradability, anti-inflammatory effects, and structural similarity to cartilage extracellular matrix, glycosaminoglycans. This review investigates recent developments in the design and application of chitosan scaffolds in cartilage tissue engineering. Various scaffold formats, including hydrogels, porous and nanofibrous structures, as well as three-dimensional printed constructs, have been shown to support the proliferation, adhesion, and differentiation of chondrocytes. On the other hand, chitosan carriers have been developed for the controlled release of growth factors, anti-inflammatory drugs, and nucleic acids, enhancing the tissue regeneration results. Engineering strategies such as chemical modification, combination with natural or synthetic polymers, and incorporation of bioactive molecules lead to improved mechanical strength, bioactivity, and immunomodulatory properties of chitosan-based scaffolds. Preclinical studies in animal models followed by initial clinical trials and subsequent production of commercial products present promising evidence of the clinical translatability of chitosan scaffolds. Recent innovations, including responsive smart scaffolds and 4D bioprinting, show that scaffolds are transitioning from static biomaterials toward dynamic structures with compatibility in physiologic environments. Overall, chitosan provides a multipurpose and promising biomaterial for cartilage regeneration. The continuation of interdisciplinary research, along with advancements in customized modeling, can accelerate the development of new-generation scaffolds, ultimately leading to improved long-term clinical outcomes.

***Keywords*:** Cartilage tissue engineering, Nanocarrier, 3D bioprinting, Regenerative medicine, Mesenchymal stem cells

Introduction

Articular cartilage is a specialized type of connective tissue that, by covering the joint surface of bones, allows smooth joint movement and distribution of mechanical loads (Gilbert and Blain, 2018). The absence of blood vessels and the low density of cartilage cells limit the intrinsic regeneration of this tissue (Dover, 2007; Hall, 2005). Traumatic injuries, aging, and degenerative disorders can lead to joint dysfunction and osteoarthritis (Chang et al., 2013). In 2020, an estimated 595 million people worldwide were living with osteoarthritis, representing about 7.6% of the global population. By 2050, this figure is expected to grow considerably (Steinmetz et al., 2023). Treatment approaches such as microfracture, mosaicplasty, and autologous chondrocyte implantation have failed to restore either the integrity or the natural structure of this tissue (Rodríguez-Merchán, 2012).

Tissue engineering utilizes cells, scaffolds, and bioactive molecules to address these limitations and to regenerate cartilage (Jeyaraman et al., 2025). In some studies, decellularized cartilage scaffolds have been developed to serve as natural matrices for various biomedical applications (Ghassemi et al., 2019). Moreover, synthetic scaffolds have also been considered for creating structures with specific properties. Among various types of biomaterials, chitosan (CS), as a natural polysaccharide, has become a focal point for researchers. With features such as biocompatibility, biodegradability, anti-

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inflammatory properties, and structural similarity to glycosaminoglycans (GAGs) in the extracellular matrix (ECM) of cartilage, chitosan offers significant potential in treating cartilage injuries (Neves et al., 2011; Sencadas et al., 2012).

Recent research suggests that chitosan-based scaffolds such as hydrogels (Maneechan et al., 2025), porous matrices (Wang et al., 2025), and nanofibrous structures (Nour-Eldeen et al., 2020) can effectively facilitate the proliferation, adhesion, and differentiation of chondrocytes, and enable the release of growth factors in a controlled manner (Zhang et al., 2021). In addition, since chitosan possesses a flexible chemical structure, its surface can be modified and combined with other polymers and nanoparticles to enhance its biological functionality (Negm et al., 2020). The results of preclinical and early clinical studies present chitosan as a promising biomaterial for the development of novel cartilage regeneration solutions. This review provides a general overview on the applications of chitosan in cartilage tissue engineering, and focuses on scaffold design, nanocarrier systems, three-dimensional (3D) bioprinting, preclinical and clinical applications, and future perspectives in the development of smart scaffolds (Figure 1).

1. Chitosan

Chitosan is a natural polymer derived from chitin found in the exoskeleton of crustaceans and cell walls of fungi, which is produced through the partial deacetylation of chitin (Croisier and Jérôme, 2013; Rinaudo, 2006).

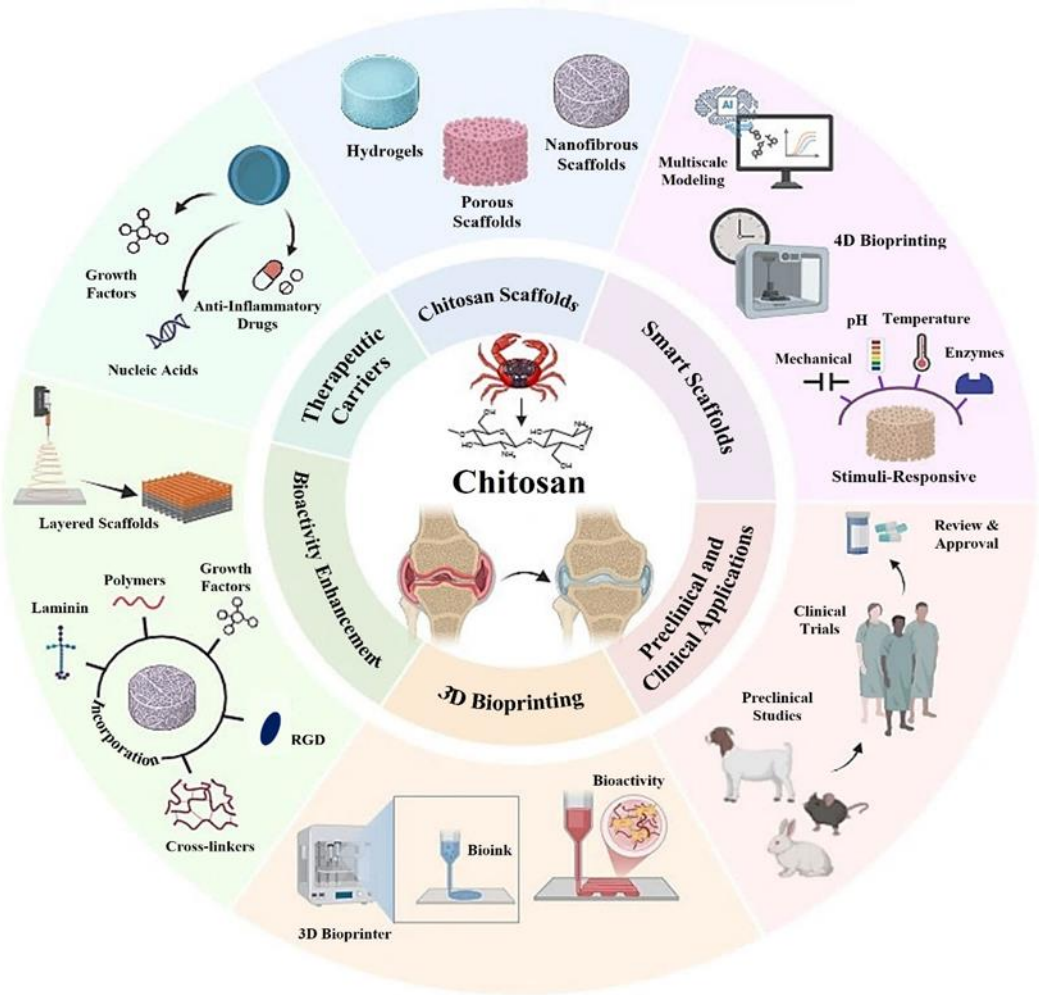

Figure 1. Schematic representation of the main applications of chitosan in cartilage tissue engineering, including the design of various types of chitosan-based scaffolds, using therapeutic carriers, enhancing scaffold bioactivity, applying 3D bioprinting technology, implementing preclinical and clinical applications, and developing smart scaffolds.

The degree of deacetylation and molecular weight of chitosan determine its properties, including mechanical strength, chemical stability, viscosity, and water solubility. The chemical structure of chitosan consists of D-glucosamine units linked by β (1→4) glycosidic bonds and randomly distributed *N*-acetylglucosamine groups. Hydrogen bonds present within the polymer network enhance stiffness and allow for the production of durable films (Leedy et al., 2011; Mars et al., 2024). pH-dependent solubility makes chitosan a suitable candidate for diverse biomedical applications (Bowman and Leong, 2006). Moreover, owing to the presence of amino and hydroxyl groups along the polymer chain, it is able to form covalent bonds with other compounds, enabling its functional modification (Rinaudo, 2006).

In recent decades, chitosan has been widely used in cartilage tissue engineering as a bioactive material (Gupta et al., 2024). The unique characteristics of chitosan that distinguish it from other bioactive materials include biodegradability, biocompatibility, hydrophilicity, antimicrobial and antifungal activity, wound-healing acceleration, and non-toxicity (Sheng et al., 2023; Shih et al., 2019). Furthermore, since this polymer can be formed into various structures such as fibers (Mirmusavi et al., 2022), sponges (Wang et al., 2025), and hydrogels (Maneechan et al., 2025), it can be utilized in a diverse range of applications. Additionally, chitosan exhibits high compatibility with various drug carriers and a high loading capacity (Obeidat and Lahlouh, 2025). This natural material can establish electrostatic interaction with GAGs and proteoglycans, and through stimulating the expression of cartilage-specific proteins, it can increase chondrogenic activity. Another distinctive characteristic of chitosan is its structural resemblance to GAGs within the ECM of cartilage, which interact with cytokines, receptors, and cell adhesion molecules (Sechriest et al., 2000; Suh and Matthew, 2000).

Cartilage tissue engineering uses chitosan as a scaffold for mimicking the ECM and as a nanocarrier for the controlled release of therapeutic agents. Altogether, these characteristics render chitosan a promising material for improving cartilage regeneration performance.

2. Chitosan-based scaffolds in cartilage tissue engineering

Scaffolds act as temporary matrices for the growth, organization, and regeneration of damaged or lost tissues. In cartilage tissue engineering, chitosan-based scaffolds have been extensively studied and developed in various formats, including hydrogels, porous/spongy structures, and nanofibrous scaffolds (Figure 2).

2.1. Hydrogels

Hydrogels are 3D networks with hydrophilic properties that, by absorbing large amounts of water and creating an environment similar to the ECM, provide a suitable platform for cellular activities, including cell adhesion, proliferation, and differentiation (Catoira et al., 2019; Souza-Silva et al., 2024). Chitosan hydrogel scaffolds are essential for supporting cell viability and promoting tissue regeneration, through their biocompatibility, biodegradability, ability to exchange nutrients and wastes, and controlled loading and release of growth factors (Catoira et al., 2019; Miri et al., 2024). Numerous studies conducted in this area have demonstrated the effectiveness of this type of scaffold. For example, a continuous porous hydrogel scaffold based on polyvinyl alcohol/chitosan (PVA/CS) succeeded in providing a proper environment for adhesion and proliferation of bone marrow mesenchymal stem cells (BM-MSCs). *In vitro* evaluations indicated high biocompatibility and enhanced differentiation of chondrogenic cells. In a rabbit cartilage defect model, some improvements were observed, including the significant quality enhancement of the regenerated tissue, increased deposition of type II collagen, and the superior integrity of the host cartilage compared to the control group (Peng et al., 2019). In another study, an injectable hydrogel containing chitosan, β-glycerophosphate, and hydroxyethyl cellulose, supported the survival and differentiation of mesenchymal stem cells into chondrocytes. Furthermore, it demonstrated the capability for the sustained release of insulin as a drug delivery model (Naderi-Meshkin et al., 2014).

Although these types of scaffolds have diverse applications in medicine, pure chitosan hydrogels usually suffer from poor mechanical strength, limited elasticity, and a high rate of *in vivo* degradation (Bhattarai et al., 2010). Their performance can be improved through chemical modifications by combining them with other reinforcing materials. For example, a study used a dynamic bioadhesive hydrogel based on chitosan functionalized by catechol and aldehyde-terminated four-arm polyethylene glycol (AF-PEG), which, together with kartogenin-containing PLGA/PEG nanoparticles, provided a 3D porous structure and a stable drug release. This system exhibited high levels of adhesion strength and compressive

modulus (~1150 kPa and ~195 kPa, respectively) and, through producing high amounts of type II collagen, achieved an organization similar to that of natural cartilage tissue in a rabbit knee cartilage defect model (Li et al., 2025).

2.2. Porous/Spongy scaffolds

Owing to their interconnected porous structure, these scaffolds allow cell penetration and exchange of nutrients and metabolic wastes (Lu et al., 2013). The presence of pores increases the contact surface area between the cells and the scaffold, leading to enhanced adhesion and cell proliferation (Madihally and Matthew, 1999). On the other hand, the higher percentage of porosity and void spaces reduces mechanical strength (Sahai et al., 2017). More porosity of the scaffold results in higher contact with biological fluids, which leads to faster biodegradation. Once the degradation and regeneration rates are synchronized, the scaffold can function effectively; otherwise, the scaffold degrades before the tissue regeneration is complete (Levengood and Zhang, 2014).

To achieve desirable performance in cartilage regeneration, chitosan porous scaffolds must be designed to provide adequate porosity for cell penetration and nutrition while maintaining sufficient mechanical strength. Wang et al. developed a chitosan/gelatin/carrageenan (CGC) composite scaffold *via* the freeze-drying method to improve cartilage regeneration. Interconnected pores with sizes ranging from 100 to 200 μm were observed, and a mechanical test demonstrated superior compressive strength and equilibrium swelling ratio of the CGC scaffold compared with the chitosan/gelatin (CG) scaffold. Primary rat chondrocytes cultured on CGC scaffolds indicated increased proliferation while maintaining their spherical morphology on days 14 and 21. Additionally, deposition of GAGs and the expression of type II collagen increased in this scaffold, and its high biocompatibility was further confirmed by subcutaneous implantation in rats (Wang et al., 2025).

2.3. Nanofibrous scaffolds

Nanofibrous scaffolds that are made from biodegradable polymer fibers, by mimicking the morphology of the ECM, are considered a promising option for cartilage tissue engineering (Shafiee et al., 2011). The potential of these scaffolds depends on parameters such as diameter adjustment, fibers composition and orientation, and the utilization of functional cells (Chiang and Jiang, 2009). These structures can be loaded with growth factor-carrying nanospheres (Schagemann et al., 2013). In certain studies, such as the design of nanofibrous chitosan microspheres, these structures have exhibited high biocompatibility, strong cellular adhesion, and the formation of 3D cartilage-like structures (Zhou et al., 2015). Various methods, such as electrospinning (Hejazi et al., 2021; Smaida et al., 2020), molecular self-assembly (Liu et al., 2011; Tamburaci et al., 2019), and phase separation (Gupte et al., 2018; Zhou et al., 2025), have been used for the production of these scaffolds. The adoption of these methods together with the 3D printing (Murab et al., 2023; Wang et al., 2009) and molding/casting (Mahapatra et al., 2019; Tamburaci et al., 2019) technologies has paved the way for the production of complex macroscopic structures that utilize 3D nanofibrous scaffolds with microporous structures. In one study, a hybrid electrospun nanofibrous scaffold containing chitosan, decellularized cartilage matrix, and polyethylene oxide was developed, which provided a biomimetic structure resembling the natural ECM. Parameter optimization using the Box-Behnken method led to increased cell adhesion and proliferation of human dermal fibroblasts (Saberi et al., 2024).

Overall, the high surface-to-volume ratio of nanofibrous scaffolds leads to increased cellular adhesion and proliferation. Additionally, the tunable porosity and fiber orientation, along with the ability to incorporate bioactive molecules, are among the key advantages of this type of scaffold (Bölgen and Vaseashta, 2016). However, challenges such as the limited solubility of chitosan and the low mechanical strength of fibers require the adoption of a proper solvent and structural enhancement using complementary materials (Bhattarai et al., 2005; Bohlouli et al., 2018). The diverse structure and composition of these scaffolds are summarized in Table 1.

3. Chitosan-based carriers for therapeutic delivery

The innovative chitosan-based carrier systems, due to their unique chemical structure and the presence of positively ionized amino functionalities, can establish electrostatic interactions with anionic biomolecules such as proteins, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA) (Bernabé et al., 2005; Danielsen et al., 2005). Consequently, they enhance the delivery of growth factors (Li et al., 2025), anti-inflammatory drugs (Wang et al., 2022), and nucleic acids (Karayianni et al., 2023). The intrinsic characteristics of chitosan, such as

biocompatibility, biodegradability, production of non-toxic byproducts, and chemical modifiability, make it an attractive candidate for both covalent and non-covalent conjugation with a wide range of functional groups or targeting ligands (Ngo et al., 2024; Nicolle et al., 2021). In a study, the use of chitosan along with positively charged amino groups facilitated drug delivery and binding with negatively charged ligands, ensuring targeted drug delivery (Khatami et al., 2021). Furthermore, the possibility of processing it in various forms, such as nanoparticles, nanocapsules, microparticles, and hydrogels, provides a suitable platform for controlled release of biological agents and therapeutic drugs, thereby increasing the precision and efficiency of these systems in regenerative medicine (Agnihotri et al., 2004; Shoueir et al., 2021; Zhang et al., 2021).

3.1. Delivery of growth factors

The short half-life of growth factors results in poor biological stability; therefore, they must be injected in high doses to achieve desirable treatment. Long-term exposure to growth factors such as the transforming growth factor beta-1 (TGF-β1) leads to adverse effects like synovial fibrosis and hypertrophic scar formation (Madry and Cucchiarini, 2014; Van Der Kraan and Van Den Berg, 2007). Chitosan carriers, through the delivery and controlled release of growth factors, can stimulate the differentiation of stem cells into chondrocytes and enhance tissue regeneration. For example, an injectable hydrogel based on crosslinked thiolated chitosan and carboxymethyl cellulose was developed as a TGF-β1 carrier. This hydrogel, with an elastic modulus of approximately 2.5 kPa, high structural stability, and gradual release (approximately 18.6% in 21 days), enhanced the chondrogenic growth and differentiation of BM-MSCs, resulting in effective, high-quality repair after eight weeks in a rat full-thickness knee cartilage defect model (Zhang et al., 2021). Additionally, in core-shell microspheres, kartogenin was loaded into the core with high efficiency, while PDGF-BB was incorporated onto the shell. Subsequently, they were combined with a chitosan/silk fibroin matrix. This thermo-responsive hydrogel, having a 6 kPa elastic modulus and suitable mechanical strength, enabled the scheduled release of both factors over several weeks. Under *in vitro* condition, this system demonstrated strong potential for endogenous cartilage repair by positively affecting BALB/c 3T3 cells and maintaining the chondrocyte growth and phenotype (Min et al., 2022).

3.2. Delivery of anti-inflammatory drugs

In osteoarthritis, the secretion of cytokines and chemokines by chondrocytes and synoviocytes, along with the production of pro-inflammatory cytokines and matrix-degrading enzymes by synovial fibroblasts, lead to inflammation and joint degeneration (Hou et al., 2017). Anti-inflammatory compounds such as dexamethasone (García-Couce et al., 2022) and curcumin (Talouki et al., 2025) alleviate joint inflammation and create a favorable environment for cartilage repair. Chitosan-based carriers reduce systemic side effects by enhancing the local delivery of anti-inflammatory drugs. In a study on the treatment of osteoarthritis, an injectable temperature-sensitive Pluronic F-127/chitosan-based hydrogel was developed for the controlled release of dexamethasone. The addition of chitosan and the tripolyphosphate (TPP) crosslinking agent changed the structure and slowed the drug release rate. These hydrogels were biocompatible, and in a rat model, prolonged drug retention in the joint was observed compared to conventional injection. This approach was proposed as a promising method for long-term delivery of the drug (García-Couce et al., 2022). Furthermore, Valentino et al. designed a thermo-sensitive intra-articular hydrogel based on Pluronic F-127 and hyaluronic acid, containing chitosan-hydroxytyrosol nanoparticles to treat osteoarthritis. This system, under pseudo-inflammatory condition in human chondrocytes, significantly decreased pro-inflammatory cytokines (TNF-α and IL-6), increased activity of the antioxidant enzyme SOD2, reduced cellular senescence, and inhibited the expression of cartilage-degrading genes (Valentino et al., 2022).

3.3. Delivery of nucleic acids

Due to the instability of nucleic acids under *in vivo* condition, it is essential to use gene carriers for their delivery (Liang et al., 2020; Rajendrakumar et al., 2017). Non-viral carriers such as chitosan, due to their simple production, low immunogenicity, and high positive charge, emerge as promising options for loading, cellular uptake, and endosomal escape of nucleic acids with negative charge during transfection (Bez et al., 2019; Cui et al., 2019; Jiang et al., 2017; Nguyen et al., 2019). Chitosan-based gene delivery systems have a wide range of applications in gene therapy, including the transfer of plasmid DNA (pDNA), small interfering RNA (siRNA), microRNA (miRNA), short hairpin RNA (shRNA), and the CRISPR-Cas9 genome-editing system (Cao et al., 2019). For instance, researchers designed chitosan–DNA nanoparticles containing a

plasmid encoding the interleukin-1 receptor antagonist (IL-1Ra), which significantly reduced cartilage lesions in a rabbit osteoarthritis model without adverse effects, thereby inhibiting the inflammatory pathways and protecting the ECM (Zhang et al., 2006).

Despite numerous advantages of chitosan, the relatively low pKa of its amino groups (6.0-6.5) leads to low solubility in physiological condition, which demands applying some modifications. Targeted delivery, cellular uptake, and endosomal protection can be enhanced through chemical modification of chitosan and its combination with other materials (Chuan et al., 2019; Qindeel et al., 2019; Wang et al., 2020). In this regard, Moghadam et al. enabled the specific targeting of chondrocytes by modifying the chitosan nanoparticles with chondroitin sulfate (ChS). These nanoparticles, after loading with a plasmid containing a reporter gene (CAG-GFP plasmid), exhibited a size of 200-300 nm and maintained their stability in physiological-like condition, and demonstrated superior transfection efficiency and gene expression *in vitro* compared to simple chitosan. In addition, expression of the *MMP13* gene was effectively suppressed in cartilage cells after being loaded with *MMP13* siRNA (Moghadam et al., 2022). The present findings indicate that the utilization of chitosan for direct gene delivery to the joint is an effective approach in arthritis gene therapy, and it can pave the way for the development of specialized advanced treatments for cartilage regeneration.

4. Engineering strategies to enhance the bioactivity of chitosan scaffolds

Modern technologies have enabled precise control over various parameters including molecular weight, degree of deacetylation, and the physicochemical structure of chitosan. These modifications enable the adjustment of parameters such as scaffold strength, porosity, and degradation rate according to special requirements of chondrocyte differentiation and effective integration within the biological environment (Berger et al., 2004; Chicea and Nicolae-Maranciuc, 2024).

Chitosan composite scaffolds have enhanced their mechanical and biological properties by combining bioactive materials and natural/synthetic polymers. Mimicking the ECM and promoting osteochondral regeneration can be enhanced by adding materials such as chondroitin sulfate, collagen, hyaluronic acid, and nano-hydroxyapatite or magnesium-doped hydroxyapatite (Henson et al., 2012; Intini et al., 2022; Yu et al., 2021; Zhang et al., 2024). Similarly, the combination of chitosan and gelatin, polylactic acid (PLA), and polyvinyl alcohol can enhance the strength and viscoelastic behavior of the scaffold to be utilized in movable joints (Wu et al., 2020).

The controlled release of chondrogenic growth factors such as TGF-β1, BMPs, IGF-1, and FGF-2 within the scaffold *via* encapsulation or surface conjugation can improve the bioactivity of chitosan scaffolds, so that the recruitment of stem cells and their differentiation into chondrocytes is promoted. The chemical structure of chitosan acts as an effective carrier for the spatial and temporal regulation of differentiation pathways through ionic interactions between chitosan and proteins (Jelodari et al., 2022; Rawojć et al., 2025; Zein et al., 2019). Additionally, the loading of drugs such as ursolic acid or norfloxacin, which have anti-inflammatory and antimicrobial properties, respectively, can aid tissue healing after surgery and prevent infection. Furthermore, ursolic acid, as an immunomodulator, enhances the likelihood of tissue regeneration and integration by changing macrophages toward the reparative M2 phenotype (Faccendini et al., 2020; Yu et al., 2021).

To synchronize the degradation of the chitosan scaffold and the cartilage regeneration process, one can adjust the degree of deacetylation, the type of crosslinkers such as genipin and glutaraldehyde, or combine it with other polymers. Optimizing these parameters helps maintain scaffold stability in the initial stages of cartilage repair (Asghari Sana et al., 2017; Berger et al., 2004). Moreover, surface functionalization with peptides such as RGD (arginine-glycine-aspartic acid) and conjugation of ECM-derived proteins such as collagen, fibronectin, and laminin, increase cell adhesion and enhances the interaction between the scaffold and cells. These approaches emphasize the importance of accurately adjusting biochemical microenvironments to more closely mimic the native cartilage niche (Jelodari et al., 2022).

Physical strategies such as nanotopographic modification, the use of electrospun nanofibers, and micropatterned surfaces enhance anisotropy and matrix deposition by directing cell orientation, gene expression, and cell migration, as well as synergizing with biochemical signals. These strategies are essential for regenerating the zonal architecture of articular cartilage (McCullen et al., 2012; Zheng et al., 2025). Furthermore, elastic and compressible chitosan scaffolds can be created by

modulating the hydrogen bonding, which can be used for minimally invasive delivery (Fan et al., 2021). Using 3D approaches such as electrospinning and freeze-drying can also allow for designing layered or gradient scaffolds, which enhance the cellular organization and tissue regeneration by mimicking the cartilage zones and the osteochondral interface, making them promising candidates for repairing complicated defects (Rawojć et al., 2025).

Through interacting with some stimuli such as enzymes, pH, and mechanical stress, smart scaffold systems can adjust features such as drug release or structural stiffness *in situ* and adapt to condition changes. Chitosan is a suitable platform for these applications, since it is enzymatically degradable and pH-sensitive (McCarthy et al., 2021; Yurtsever et al., 2022). For example, mechanoresponsive chitosan scaffolds are tailored to load-bearing environments by changing their properties with pressure. In a study, scaffolds with high compressive strength and high elastic recovery (around 80% recovery after five times applying pressure) were created without needing toxic crosslinking agents (Silvestro et al., 2021). This was achieved through utilization of thermally induced phase separation (TIPS), freeze-gelation, accompanied by establishing optical crosslinks. In advanced mechanosensitive approaches, microcapsules or microstructures are utilized that can release bioactive compounds such as TGF-β3 in response to mechanical forces or oscillatory fluid flow (Lima et al., 2007).

Thanks to recent advancements in the field of materials engineering, chitosan has been promoted to a structural bioactive platform, which enables the design of new-generation scaffolds compatible with the complex requirements of cartilage regeneration.

5. 3D bioprinting and applications of chitosan in cartilage tissue engineering

3D bioprinting is an advanced technology in regenerative medicine that allows for the fabrication of complex, natural tissue-like structures by stacking cells and biomaterials layer by layer (Murphy and Atala, 2014; Xu et al., 2013). This method provides superior control over scaffold architecture and cell distribution, owing to its micrometer-scale precision in deposition (Zhang et al., 2017). In recent years, printable bioinks have found extensive applications in tissue engineering and drug delivery fields (Das and Basu, 2019; Taghizadeh et al., 2022). They offer a range of advantages, including accurate process control, direct encapsulation of cells and biomolecules, and the ability to be modified post-print, and the high resolution in fabrication of organs (Knowlton et al., 2017; Zhang et al., 2016).

In 3D bioprinting, a suitable bioink must have three key sets of features: firstly, features that influence proper printability, including optimal viscosity, ability to form crosslinks, and post-print structural stability; secondly, features that influence bioactivity, defined by factors such as biocompatibility, supporting cell survival, promoting cell adhesion and proliferation, and sufficient porosity; and thirdly, features that influence mechanical properties, encompassing mechanical strength approximating that of native tissue, good flexibility, and biodegradability (Parak et al., 2019). Enhancing the physical strength of the structure requires crosslinking within the bioink through chemical, physical, or enzymatic processes (Hospodiuk et al., 2017).

Due to limitations of synthetic polymers such as low biocompatibility and poor cell adhesion, natural polymers are the preferred candidates for bioprinting (Pereira and Bártolo, 2015; Wang et al., 2016). Chitosan, as a biocompatible polycationic polymer, with physiological stability and suitable viscosity, supports cell growth and differentiation (Sahranavard et al., 2020). Scaffolds made of chitosan mimic the ECM and enable cell/matrix interaction, material exchange, and appropriate immune response (Derakhshanfar et al., 2018). These scaffolds maintain their biocompatibility and bioactivity, even after post-print modifications (Chimene et al., 2020; Huang et al., 2016). The three primary bioink printing methods include extrusion, laser printing, and inkjet printing, among which, the extrusion method is more common for chitosan-based inks (Wu et al., 2018). Suitable bioinks for micro-extrusion should exhibit viscosities ranging from 30 mPa·s up to over 6×10^7 mPa·s. In these inks, the concentration of additives and the density of crosslinks can control mechanical characteristics and *in vivo* degradation rates (Demirtaş et al., 2017; Murphy and Atala, 2014). In the 3D-printing process, shape fidelity (as in retention of the precise design geometry) is of utmost significance, as it ensures the stability of the ultimate structure. For this purpose, a study added hydroxyethyl cellulose to the chitosan formulation to improve the printability and structural precision by increasing ink viscosity (Afra et al., 2024).

Li et al. conducted a study to design a hybrid system for cartilage regeneration that consisted of PCL and chitosan hydrogel 3D-printed scaffold. This

scaffold was loaded with SMSCs. A tetrahedral framework nucleic acid (TFNA) was used as a bioactive DNA nanostructure to enhance repair. PCL was responsible for providing the mechanical strength and maintaining the 3D structure, while chitosan hydrogel provided a moist, biocompatible environment for cells. Due to its positive charge, it was capable of absorbing and retaining the TFNA injected into the joint. *In vitro* results indicated that TFNA has effectively entered the SMSCs and increased the expression of cartilage genes such as *COL2A1* and *ACAN*. In a rabbit cartilage defect model, outcomes included the regeneration of a tissue similar to healthy cartilage, increased glycosaminoglycan and type II collagen contents, and improved mechanical properties (Figure 3). This study exemplifies the synergy among 3D printing technology, bioactive hydrogels, and nucleic acid nanostructures, in cartilage tissue engineering (Li et al., 2021). In another study, researchers used 3D-printed alginate-chitosan scaffolds loaded with insulin to regenerate damaged knee cartilage. These scaffolds, with their porous architecture and high mechanical strength, enabled controlled insulin release and promoted the differentiation of MSCs into chondrocytes. According to the results, in addition to high biocompatibility, this system leads to increased expression of key chondrogenic genes, including *COL1A1*, *COL2A1*, *SOX9*, and *ACAN*, and they can accelerate the tissue regeneration process (Jahani et al., 2025).

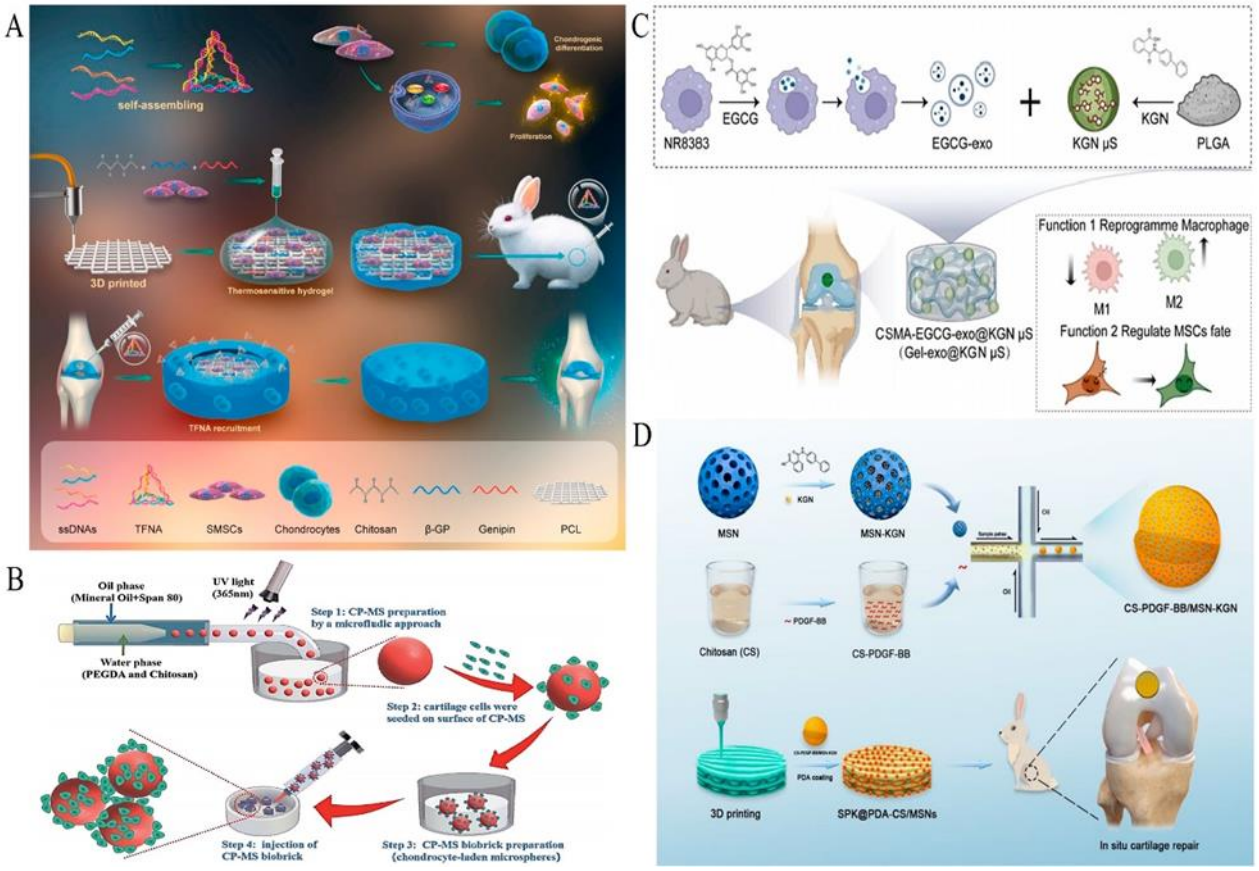

Figure 2. Illustrative examples of chitosan-based scaffolds in cartilage tissue engineering: 3D-printed polycaprolactone (PCL) scaffold combined with chitosan hydrogel and synovial mesenchymal stem cells (SMSCs) (A) (Reproduced from Li et al., 2021, licensed under CC BY-NC-ND 4.0); injectable chitosan/poly(ethylene glycol) diacrylate hydrogel as chondrocyte carriers (B) (Reproduced from Lin et al., 2020, licensed under CC BY 3.0); chitosan methacryloyl hydrogel containing immunomodulatory exosomes and KGN microspheres (C) (Reproduced from Liang et al., 2025, licensed under CC BY-NC-ND 4.0); bioactive porous implant with chitosan/mesoporous silica microspheres and sequential release of platelet-derived growth factor BB (PDGF-BB) and KGN (D) (Reproduced from Yuan et al., 2022, licensed under CC BY 4.0). Each of these scaffolds presents a distinct approach for enhancing cartilage regeneration.

Table 1. Chitosan-based scaffolds and their applications in cartilage tissue engineering.

Scaffold composition	Scaffold type	Fabrication method	Cell type / Animal model	Results	Reference
Chitosan, silk fibroin, *Aloe vera* (CS/SF/AV)	Hydrogel scaffold	Lyophilization (freeze-drying)	Human primary chondrocyte cell line	CS40/SF/AV hydrogel scaffold with high porosity, suitable compressive strength, controlled swelling, and gradual degradation provided a biocompatible environment for cell adhesion and proliferation of chondrocytes *in vitro*.	(Maneechan et al., 2025)
Catechol-functionalized chitosan, PLGA, PEG, KGN (CS-HCA@PLGA-PEG@KGN)	Injectable hydrogel scaffold	Dynamic covalent bond formation	BM-MSCs / rat knee joint cartilage defect model	The injectable bioadhesive hydrogel with controlled KGN release enhanced adhesion, proliferation, and chondrogenic differentiation of BM-MSCs *in vitro*, while supporting cartilage regeneration *in vivo* through type II collagen deposition. However, its limited mechanical strength under wet condition remains a significant limitation.	(Li et al., 2025)
GelMA, glycol chitosan (GelMA-GC)	Injectable hydrogel scaffold	UV-induced photocrosslinking (covalent bonding)	BM-MSCs / bovine cartilage (*ex vivo*)	The photocrosslinkable injectable GelMA-GC hydrogel with strong adhesion, enhanced proliferation, migration, and chondrogenic differentiation of BM-MSCs, accompanied by type II collagen deposition *in vitro*, was developed. In an *ex vivo* cow cartilage model, this hydrogel adhered to the tissue properly and provided suitable mechanical stability.	(Paul et al., 2023)
Chitosan, gelatin, carrageenan (CGC)	Porous scaffold	Lyophilization	Rat primary chondrocytes	The porous solid CGC scaffold (porosity 100–200 µm) supported chondrocyte proliferation and phenotype maintenance for up to 21 days, and was accompanied by increased type II collagen expression and GAG deposition.	(Wang et al., 2025)

Scaffold composition	Scaffold type	Fabrication method	Cell type / Animal model	Results	Reference
Agarose, chitosan, collagen, gelatin (AG-CH-COL-GEL)	Porous scaffold	Freeze-drying	Human chondrocytes	The AG-CH-COL-GEL biocomposite porous scaffold (pore size 88–278 μm) exhibited high water uptake, suitable mechanical properties (elastic modulus 44.91–201.77 kPa), and excellent biocompatibility, which increased the proliferation of human chondrocytes and aggrecan gene expression.	(Chonanant et al., 2024)
PCL, chitosan, MWCNTs	Nanofibrous scaffold	Electrospinning	Chondrocytes	The nanocomposite scaffold simultaneously improved mechanical properties (strength and flexibility), biological performance (chondrocyte adhesion and proliferation), and structural characteristics (porosity and hydrophilicity).	(Mirmusavi et al., 2022)
Chitosan, poly (vinyl alcohol)	Nanofibrous scaffold	Electrospinning	Ad-MSCs	The nanofibrous scaffold, by providing an ECM-like environment, enhanced adhesion and proliferation of Ad-MSCs and promoted their chondrogenic differentiation.	(Nour-Eldeen et al., 2020)
PLGA fiber arrays, citric acid-modified chitosan, decellularized cartilage matrix	Hydrogel–fibrous scaffold	Electrospinning and chemical crosslinking	Chondrocytes / rabbit animal model	The biomimetic hydrogel–fibrous scaffold enhanced mechanical and biological properties, promoted GAG and type II collagen production with improved chondrocyte function, and in a rabbit model enabled regeneration of subchondral bone and hyaline cartilage.	(Shen et al., 2021)
Porous carboxymethyl chitosan sponge with glucosamine sulfate, silk fibroin, PCL core–shell nanofibers	Porous nanofibrous sponge scaffold	Coaxial electrospinning and freeze-drying	Chondrocytes / rat femoral condyle articular cartilage defects	The biocompatible scaffold enhanced chondrocyte adhesion, spreading, and proliferation, enabled controlled drug release for up to 30 days; and, in a mouse femoral condyle defect model, improved hyaline cartilage formation with abundant type II collagen deposition and full integration with the surrounding tissue.	(Ma et al., 2025)

Abbreviations: Adipose-derived mesenchymal stem cells (Ad-MSCs), Bone marrow mesenchymal stem cells (BM-MSCs), Carboxyl-functionalized multi-walled carbon nanotubes (MWCNTs), Gelatin methacrylate (GelMA), Kartogenin (KGN), Poly (lactic acid-co-glycolic acid) (PLGA), Polycaprolactone (PCL), Polyethylene glycol (PEG)

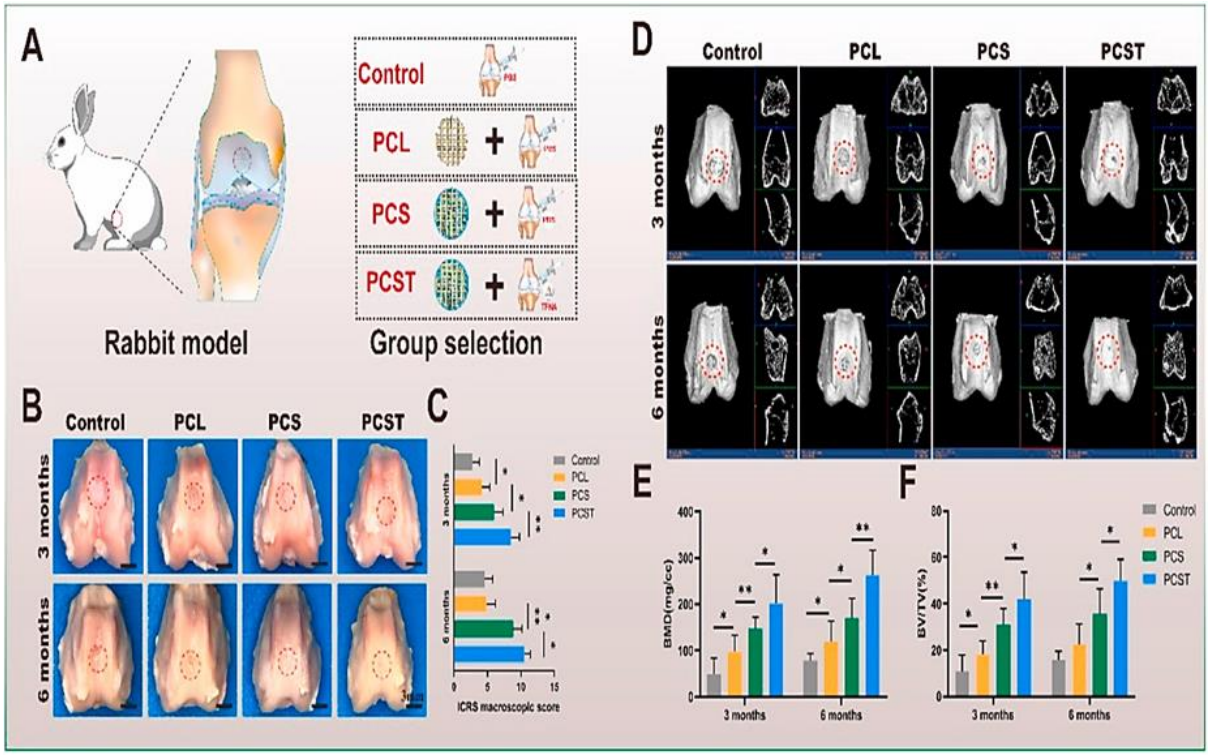

Figure 3. Assessing the performance of a 3D-printed hybrid scaffold in repairing rabbit cartilage. A schematic illustration of the tested groups (control groups, PCL, PCL+CS, and PCL+CS+TFNA) (A). Red circles represent the gross images of the regenerated tissues, 3- and 6-months post-surgery (B). Macroscopic evaluation of groups based on the International Cartilage Repair Society (ICRS) (C). 2D and 3D images of the lesion using Micro-CT (D). Quantitative results of bone mineral density (BMD) (E) and bone volume/total volume ratio (BV/TV) (F) at the lesion area. Statistical significance levels of $p < 0.05$, $p < 0.01$, and $p < 0.001$ are indicated by *, **, and ***, respectively. Reproduced from Li et al., 2021, licensed under CC BY-NC-ND 4.0.

Chitosan-based bioink 3D printing faces various challenges, including the dependence of printability characteristics on molecular weight, degree of deacetylation, pH, and viscosity, which affect the precision, strength, and biocompatibility of the structures (Morris et al., 2017; Roy et al., 2017; Tirella et al., 2009). Furthermore, issues such as low gelation rate, nozzle blockage, temperature and light limitations, and post-print structural variations necessitate optimization of both bioink formulations and printing techniques (Hospodiuk et al., 2017; Kohut et al., 2007; Sahranavard et al., 2020).

6. Preclinical and clinical applications of chitosan scaffolds

Due to their biocompatibility, controllable degradability, similarity to GAGs, and anti-inflammatory properties, chitosan scaffolds stand out as a promising option in cartilage tissue engineering (Aranaz et al., 2021). Preclinical studies have demonstrated preliminary safety and efficacy in animal models, paving the way for clinical trials. These trials facilitate the translation of these scaffolds into standard therapies by evaluating their effectiveness and safety.

Animal models play a pivotal role in the development of cartilage repair. Small animal models such as rabbits and rodents are suitable for preliminary investigations. However, due to anatomical differences from humans, they have a limited translational value (Frisbie et al., 2006; Lind et al., 2008). Conversely, larger models with better resemblance to humans are more significant in evaluating the effectiveness and preclinical safety, particularly in full-thickness lesions and load-bearing regions. However, their adoption faces limitations such as high treatment costs and specialized care requirements. Among large animals, horses are the only model capable of accommodating

defects comparable in size to those in humans, while dogs, goats, and pigs present smaller defects. Moreover, the genetic diversity of these animals can render outcomes variable and necessitate the use of larger sample sizes. Ultimately, model selection must be carried out based on the research question, economic implications, and anatomical and biomechanical characteristics (Ahern et al., 2009; Chu et al., 2010).

Various animal models were used in cartilage regeneration studies. For example, to address the insolubility of kartogenin, a CS-KGN-MA compound was synthesized by linking KGN to chitosan and modifying it with methacrylate. *In vitro* results demonstrated biocompatibility and the ability to preserve the chondrogenic potential of chondrocytes, and in the rabbit model, led to the effective repair of cartilage defect and improved the ICRS, BV/TV, and BMD indices (Ding et al., 2025). Moreover, Kim et al. designed a chitosan-gelatin hydrogel containing methotrexate and melanin. This hydrogel, once exposed to near-infrared (NIR) laser radiation, simultaneously enabled chemo-photothermal therapy, accelerated the drug release, reduced inflammation, and repaired the joint in a mouse arthritis model. Furthermore, the Micro-CT results showed the restoration of joint characteristics to a healthy condition (Kim et al., 2022).

Delvaux et al. used a sheep arthroscopic model to examine the effect of reinforcing meniscal sutures with freeze-dried chitosan combined with autologous platelet-rich plasma (PRP). In 22 adult sheep with unilateral radial meniscal lesions, the chitosan/PRP combination remained stable at the lesion area, induced immune cell uptake, and over a 6-month period enhanced the mechanical and structural quality of the meniscus and cartilage. While the treatment safety was similar to other groups, this combination was able to limit the cartilage degeneration and emerged as a promising option for meniscus repair (Delvaux et al., 2025). Additionally, in another study, an injectable hydrogel was designed to mimic natural tissue, constructed from silk fibroin, modified chitosan, and cartilage ECM after decellularization. This biocompatible hydrogel enhanced cell adhesion and proliferation through its network structure and induced the chondrogenic differentiation of BM-MSCs by significantly increasing the expression of the *SOX9*, *ACAN*, and *COL2A1* chondrogenic genes. Moreover, this hydrogel fully repaired the cartilage defects in the rabbit trochlear groove and improved the matrix regeneration and expression of cartilage components in the femoral condyle of goats (Wan et al., 2025).

Despite the valuable *in vitro* and preclinical results, the clinical translation of this technology encounters various challenges, such as instability during scaffold fabrication, differences among product batches, the probability of immune response due to animal origin, and mechanical weakness in real-life load-bearing conditions of the joint. Furthermore, the commercialization of the product poses serious obstacles and regulatory requirements such as guaranteeing sterility, contamination control, and adherence to good manufacturing practice (GMP) (Rawojć et al., 2025; Sancho-Tello et al., 2017). Despite these challenges, some studies progressed to the clinical phase. In a clinical trial study conducted on 39 patients with talus osteochondral lesions, the chitosan scaffold led to significant functional improvement (based on the AOFAS scale) and pain alleviation (based on the VAS scale) up to 12 months after the surgery, and these results remained stable at the 24-month follow-up. Moreover, the MOCART evaluation demonstrated a satisfactory quality of cartilage repair (Akmeşe et al., 2020). Similarly, a Level III clinical study conducted in 2022 evaluated the efficacy of a liquid chitosan scaffold in 32 patients with osteochondral lesions of the knee condyle. Evaluations based on Lysholm and IKDC functional scales and VAS scale indicated significant functional improvement and pain reduction up to 12 months after surgery, which remained stable after 24 months. Ultimately, the satisfactory quality of the cartilage repair was confirmed by the MOCART scoring system (Akmeşe et al., 2022).

BST-CarGel® is a commercial chitosan-based polymer scaffold that, as a Microfracture complement, promotes cartilage regeneration by stabilizing the blood clot. Clinical observations showed that this product enhances both the quantity and quality of repair tissue resembling hyaline cartilage, and it is also more cost-effective than Microfracture alone in the short-term, while reducing the risk of treatment failure (Chevrier et al., 2007; Shive et al., 2006; Stanish et al., 2013). A clinical study was conducted on 12 patients with medial femoral condyle lesion. In this study, the application of Microfracture, accompanied by either BST-CarGel® or Hyalofast, led to significant improvement in terms of WOMAC and AMADEUS clinical scores and lesion filling. These results indicate that adding scaffolds to Microfracture can enhance the quality of cartilage repair while they still

require long-term investigations (Aytekin and Esenyel, 2021).

7. Future perspectives in the design of smart chitosan-based scaffolds

During recent years, the cartilage tissue engineering field has been moving toward the development of multifunctional, smart, and personalized scaffolds, where chitosan finds a special status, due to being a biocompatible and tunable biopolymer (Rawojć et al., 2025). As one of these newly emerging approaches, one can name smart stimuli-responsive chitosan scaffolds, which can react to temperature changes, enzymatic activity, pH variations, or mechanical stress, and control the release of drugs, rate of degradation, or stiffness, in a dynamic manner (Yang et al., 2024; Tian and Liu, 2023). β-Glycerophosphate-modified chitosan hydrogels are an example of this technology, whose state shifts from sol to gel by body temperature, and have been used for minimally invasive *in situ* delivery of chondrogenic agents (Chenite et al., 2000).

Adding bioactive materials such as exosomes to chitosan systems can influence cellular behavior and cartilage regeneration. To confirm this, a research study was conducted to observe the behavior of a two-layered chitosan cryogel loaded with chondrocyte exosomes. Results indicated that it led to increased proliferation and cell migration, and consequently, improved targeted regeneration of cartilage and osteochondral tissues (Nikhil and Kumar, 2022). Moreover, computational modeling with artificial intelligence (AI) has also been considered a modern instrument for the prediction of the optimal porosity composition, degradation kinetics, and mechanical properties, and it can adapt the scaffold design to the defect anatomy of each patient (Omigbodun, 2025). Furthermore, the incorporation of the time dimension in 3D printing and the emergence of 4D bioprinting have enabled the fabrication of dynamic structures that can be altered after installation (shape-memory behavior) (Agarwal et al., 2023; Alanazi et al., 2025).

In the future, the production of personalized scaffolds by means of accurate magnetic resonance imaging (MRI) and through customized printing, using patients' autologous cells, will pave the way for the development of personalized treatments, thereby reducing the risk of immune rejection (Kilian et al., 2021). Generally, such innovations in chitosan scaffolds point to the paradigm shift from static biomaterials to patient-specific dynamic bioactive systems, which can effectively enhance cartilage repair.

Conclusion

Chitosan has emerged as a promising multipurpose biomaterial in cartilage tissue engineering. Its intrinsic features, including biocompatibility, biodegradability, structural similarity to GAGs, and anti-inflammatory activity, provide a strong basis for the development of scaffolds and therapeutic delivery systems. In the past two decades, a diverse range of chitosan-based platforms, including hydrogels, porous and nanofibrous scaffolds, nanocarriers, and bioinks, have exhibited significant potential in supporting chondrocyte proliferation, differentiation, and ECM deposition.

Despite its numerous advantages, this material suffers from various limitations, including low mechanical strength, low solubility and elasticity, high degradation rate, challenging 3D-printability, production instability, and potential of immune response due to its animal origin. Engineering strategies such as chemical modification, incorporation of bioactive molecules, and combination with natural or synthetic polymers have led to improved mechanical strength, bioactivity, and immunomodulatory properties of chitosan scaffolds. The combination of smart and responsive attributes, such as shape-memory behavior and controlled release of growth factors or nucleic acids, has changed the paradigm from static biomaterials to dynamic, bioactive systems, which are capable of becoming compatible with the physiological environment.

The safety and efficacy of chitosan scaffolds have been confirmed by preclinical animal model investigations, and the initial clinical trials of commercial products like BST-CarGel® were indicative of promising evidence regarding their clinical translatability. Nevertheless, multiple challenges such as heterogeneity in the process of scaffold development, limited mechanical performance in real-life joint loading conditions, and regulatory requirements for mass production still remain.

In the future, the collaborations of AI, computational modeling approaches, and advanced bioactive technologies can converge to accelerate the development of multipurposed patient-specific scaffolds. Personalized approaches that include accurate imaging, use of autologous cells, and bioactive modifications can ultimately provide patient-specific treatments tailored to individual

needs, which reduce the risk of immune rejection and long-term consequences.

Overall, chitosan scaffolds are considered a transformative achievement in cartilage tissue engineering. Interdisciplinary research and clinical validations need to remain continuous in order to fully realize the true capacity of these scaffolds and to translate these innovations into reliable, affordable, and accessible treatments for cartilage repair.

Conflict of Interests

The authors declare no competing interests.

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