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Chitosan-based biomaterials for hydrogels in bone tissue regeneration

Biomateriales a base de quitosano para hidrogeles de regeneración del tejido óseo

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Abstract

Bone regeneration remains a major challenge in biomedical engineering, particularly due to the clinical limitations of autologous and allogeneic grafts. In this context, chitosan-based hydrogels (CHs) have emerged as promising platforms due to their biocompatibility, gelation capacity, structural porosity, and ease of functionalization. This review presents a comprehensive analysis of the biological fundamentals of bone regeneration, the physicochemical properties of chitosan, and the hydrogel fabrication techniques employed to emulate the bone extracellular matrix. Alternative sources of chitin (crustaceans, insects, fungi) are examined, along with deacetylation methods (chemical, enzymatic, microwave-assisted, and deep eutectic solvents) and their impact on the degree of deacetylation (DD), molecular weight, and mechanical behavior of the material. Advanced approaches such as electrospinning, freeze-drying, and 3D bioprinting are described, highlighting their influence on porous architecture, controlled release of osteoinductive factors, and cell viability. Current challenges are also identified, including limited solubility, lack of standardization of structural parameters, and the clinical scalability of CHs, proposing research lines oriented toward personalized therapies and multifunctional bioactive platforms. Furthermore, application perspectives in osteochondral regeneration, targeted drug delivery, and tissue bioprinting are discussed, reinforcing the potential of chitosan as a strategic biopolymer in regenerative medicine.

Keywords: hydrogels, chitosan, chemical properties, mechanical properties, tissue engineering.

Resumen

La regeneración ósea sigue siendo un gran desafío en la ingeniería biomédica, debido principalmente a las limitaciones clínicas de los injertos autólogos y alogénicos. En este contexto, los hidrogeles basados en quitosano (CHs) han surgido como plataformas prometedoras gracias a su biocompatibilidad, capacidad de gelación, porosidad estructural y facilidad de funcionalización. Esta revisión ofrece un análisis exhaustivo de los fundamentos biológicos de la regeneración ósea, las propiedades fisicoquímicas del quitosano y las técnicas de fabricación de hidrogeles empleadas para emular la matriz extracelular ósea. Se examinan fuentes alternativas de quitina (crustáceos, insectos y hongos), junto con los métodos de desacetilación (químicos, enzimáticos, asistidos por microondas y con disolventes eutécticos profundos) y su impacto en el grado de desacetilación (DD), el peso molecular y el comportamiento mecánico del material. Se describen enfoques avanzados como el electrohilado, la liofilización y la bioimpresión 3D, destacando su influencia en la arquitectura porosa, la liberación controlada de factores osteoinductivos y la viabilidad celular. También se identifican los retos actuales como la solubilidad limitada, la falta de estandarización de parámetros estructurales y la escalabilidad clínica de los CHs) y se proponen líneas de investigación orientadas hacia terapias personalizadas y plataformas bioactivas multifuncionales. Además, se discuten las perspectivas de aplicación en regeneración osteocondral, liberación dirigida de fármacos e impresión de tejidos, reforzando el potencial del quitosano como biopolímero estratégico en la medicina regenerativa.

Palabras clave: hidrogeles, quitosano, propiedades químicas, propiedades mecánicas, ingeniería de tejidos

1 Introduction

The need to restore lost bone tissue remains a significant challenge in biomedical engineering and clinical practice. Although autologous and allogeneic bone grafting procedures are effective, they are associated with significant complications, such as donor site morbidity, risk of cross-infection, and limited material availability (Ansari, 2019). In this context, tissue engineering has emerged as a promising strategy, proposing the design of biomimetic scaffolds capable of emulating the extracellular matrix (ECM) while simultaneously promoting in situ bone regeneration (De León-Oliva *et al*., 2023; Rondón *et al*., 2025).

Among the candidate biomaterials, chitosan-based hydrogels stand out due to their remarkable biocompatibility, hydrophilic nature, and ability to form highly porous three-dimensional networks—key properties that support cell adhesion, migration, and differentiation (Aguilar *et al*., 2019; Kim *et al*., 2023). Chitosan, a polysaccharide obtained by the deacetylation of chitin derived from marine waste, insects, and fungi, contains free amino groups that confer a positive charge under physiological conditions. This facilitates electrostatic interactions with ECM proteins and promotes bone mineralization (Aranaz *et al*., 2021). Furthermore, chitosan can be chemically modified to tune its solubility, responsiveness to stimuli, and degradation profile, thereby expanding its applicability in advanced therapeutic approaches (Novikov *et al*., 2023).

Literature reports indicate that chitosan hydrogels—whether injectable, self-healing, or structured via 3D bioprinting—enable the controlled incorporation of osteoinductive factors, hydroxyapatite nanoparticles, or osteoprogenitor cells, thus enhancing bone neogenesis and integration with host tissue (Li *et al*., 2023; Lazaridou *et al*., 2022). Their porous architecture and high-water content (>90%) recreate a physiological microenvironment that facilitates nutrient and metabolite exchange—an essential feature in critical-size bone defects where vascularization is often compromised (Nallusamy & Das, 2021).

However, the versatility of chitosan strongly depends on its source and degree of deacetylation (DD). These parameters influence its molecular weight, crystallinity, and mechanical and biological properties (Huq *et al*., 2022). Therefore, standardization of purification and characterization methods is essential to ensure reproducibility and clinical safety. In this context, the present review article aims to:

1. Critically analyze the sources, extraction methods, and chemical modifications of chitosan, emphasizing their impact on physicochemical properties relevant to bone regeneration.
2. Summarize recent advances in the design and fabrication of chitosan hydrogels—including electrospinning, lyophilization, and 3D printing techniques—and their relationship with cellular responses and new bone formation.
3. Identify knowledge gaps and technical challenges that still hinder the clinical translation of these systems, proposing future research directions aligned with trends in personalized medicine and biofabrication.

The discussion is structured into sections covering (i) the biological fundamentals of bone regeneration, (ii) the intrinsic characteristics of chitosan, (iii) hydrogel synthesis methodologies, and (iv) their main biomedical applications. This comprehensive overview aims to help researchers and clinicians assess the real potential of these biomaterials and guide the development of more effective therapeutic strategies.

2 Methodology

2.1 Search strategy and databases

A systematic literature search was conducted following the PRISMA guidelines for reviews (Page *et al*., 2021; Moher *et al*., 2009). Search was performed between January and March 2025 across the following electronic databases: PubMed/MEDLINE, Scopus, Web of Science, ScienceDirect, Wiley Online Library, Royal Society of Chemistry, MDPI, IEEE Xplore, SciELO, RedALyC, and Google Scholar. Additionally, indexed conference proceedings and the reference lists of key articles were screened to identify further relevant literature.

Search queries used Boolean operators and truncation; a representative example was: (bone regeneration OR "bone tissue engineering") AND (chitosan OR "chitosan hydrogel*" OR quitosano) AND (scaffold* OR hydrogel* OR biomaterial*).

2.2 Inclusion and exclusion criteria

Table 1 summarizes the inclusion and exclusion criteria used in this review.

2.3 Data extraction and organization

For each eligible study, the following information was recorded:

- Source and type of chitin/chitosan (crustacean, insect, fungus)
- Degree of deacetylation (DD) and molecular weight
- Hydrogel synthesis method (physical crosslinking, chemical crosslinking, self-assembly, 3D printing,

electrospinning, freeze-drying, etc.)
- Chemical properties (surface charge, solubility, crystallinity) and mechanical properties (elastic modulus, tensile strength, porosity)
- Biological model design (cell lines, animal models, growth factors, mineralization, evaluation times)
- Main outcomes and reported limitations

The reference manager Mendeley was used to filter and classify articles according to the thematic focus of this review:

a. Composition and origin of chitosan (source, DD, purity)
b. Physicochemical and mechanical properties relevant to osteogenesis
c. Hydrogel fabrication techniques and their impact on biological performance

2.4 Qualitative synthesis

Data were grouped according to the classification scheme proposed by Nallusamy and Das (2021) for hydrogels, complemented by the chemical modification taxonomy proposed by Aranaz *et al*. (2021). An analysis was conducted on the relationship between the production method, resulting properties, and preclinical outcomes. This approach allowed for identifying correlations and knowledge gaps, which will be discussed in subsequent sections.

Table 1. Inclusion and exclusion criteria of the study.

Category	Inclusion Criteria	Exclusion Criteria
Document Type	Original research articles, systematic or narrative reviews, and peer-reviewed experimental reports with full text	Abstracts without full text, non-peer-reviewed short communications, and patents
Language	English or Spanish	Other languages
Time Frame	Publications between January 1, 2004, and March 31, 2025	Publications outside this time range
Content	Studies describing chitosan-based hydrogels for bone regeneration (*in vitro*, *in vivo*, or *ex vivo*), including data on composition, synthesis, physicochemical properties, or biological performance	Studies have not focused on chitosan or are not related to bone tissue.
Quality	Clear experimental design and minimum quantitative data ($n \geq 3$)	Insufficient methodological information or duplicate studies

3 Results and discussion

3.1 Biological fundamentals of bone regeneration

Bone regeneration is a highly coordinated process that fundamentally mimics the mechanisms of embryonic skeletal development. It is considered a model of complete tissue regeneration, as the newly formed tissue preserves the original's structure and functionality (Ansari, 2019). This process is crucial for fracture healing and tissue engineering applications aimed at restoring bone volume lost due to trauma, degenerative diseases, or tumor resections.

3.1.1 Physiological process of bone regeneration

Bone regeneration (Figure 1) occurs in three sequential but interrelated phases: inflammation, new bone formation, and remodeling (Henry & Bordoni, 2023). In the inflammatory phase, tissue rupture triggers the release of cytokines and growth factors (such as TGF-β, BMPs, and PDGFs), which initiate the hemostatic cascade, followed by the recruitment of inflammatory cells to the injury site. This phase establishes a key pro-regenerative environment for the next stage. During callus formation, fibrous tissue and cartilage (soft callus) are initially produced and mineralized into immature bone (hard callus). This process critically depends on the osteogenic activity of progenitor cells and a microenvironment that provides adequate oxygenation, nutrients, and structural support.

Remodeling involves the resorption of primary bone and its replacement with mature lamellar bone, restoring the original osteonal architecture. Depending on mechanical, hormonal, and cellular factors, this process can last for months or even years (Yue *et al*., 2020).

3.1.2 Cells involved in bone regeneration

Bone tissue is highly dynamic and depends on the coordinated action of multiple cell types. Among the most relevant in bone regeneration are:

- Osteoprogenitor or osteogenic cells: Mesenchymal stem cells located in the periosteum and endosteum. They can differentiate into osteoblasts under appropriate mechanical and biochemical stimuli

(Henry & Bordoni, 2023).

- Osteoblasts: Bone-forming cells derived from mesenchymal progenitors. They secrete osteoid matrix rich in type I collagen and play an active role in bone mineralization. They also express alkaline phosphatase and osteocalcin, key osteoblastic phenotype markers (Li *et al*., 2023).
- Osteocytes: Mature osteoblasts that become embedded in the mineralized matrix. They communicate through canaliculi and play roles in mechanotransduction, mineral homeostasis, and signaling for bone remodeling. Osteocytes are the most abundant cells in bone tissue (Nahian *et al*., 2023).
- Osteoclasts *(though not osteogenic)*: Multinucleated cells derived from the hematopoietic lineage, responsible for bone resorption. Their functional balance with osteoblasts ensures appropriate physiological bone remodeling.

The activation and coordination of these cells are regulated by systemic factors (hormones, vitamins), local signals (cytokines, growth factors), and the physicochemical microenvironment of the bone niche.

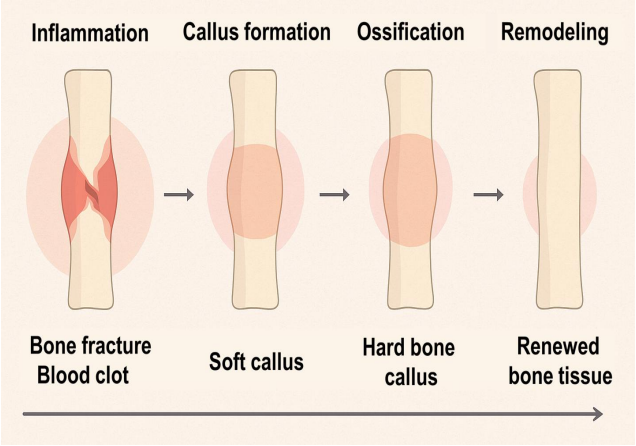

Figure 1. Physiological process of bone regeneration.

3.1.3 Composition of the bone matrix

The bone extracellular matrix (ECM) constitutes the biological scaffold that provides mechanical strength and biochemical signaling. Its composition can be divided into three main fractions:

- Organic fraction (~30% of dry weight): Predominantly composed of type I collagen (90–95% of the organic matrix), along with type V collagen, proteoglycans (such as decorin and biglycan), glycoproteins (osteonectin, osteopontin), growth factors, and bone morphogenetic proteins (BMPs) (De León-Oliva *et al*., 2023). These components mediate cell–matrix interactions and modulate cell adhesion, migration, and differentiation processes.
- Inorganic fraction (~60%): Primarily composed of hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$], which are responsible for bone mineralization and stiffness. This phase provides the tissue with its ability to withstand compressive forces.
- Water (~10%): Essential for nutrient transport, ion exchange, and maintenance of osmotic balance within the tissue.

Accurately replicating these characteristics in a biomaterial is essential for the design of functional scaffolds. For this reason, chitosan-based hydrogels—offering a hydrated polymeric matrix with tunable chemical functionality—have emerged as promising candidates to mimic the bone ECM (Aranaz *et al*., 2021; Kim *et al*., 2023).

3.2. Hydrogels as platforms for bone tissue engineering

Hydrogels have assumed a central role in designing biofunctional platforms for tissue engineering, particularly in bone regeneration. Thanks to their high water content, soft yet crosslinked structure, and capacity for chemical functionalization, hydrogels efficiently mimic the properties of the bone's natural extracellular matrix (ECM), thus facilitating cell adhesion, proliferation, and differentiation (Li *et al*., 2023; Aranaz *et al*., 2021). In the specific case of chitosan, its polysaccharide structure with primary amino groups allows a wide range of physicochemical modifications, making it a versatile material for biomedical applications.

3.2.1 Functional characteristics of hydrogels

Hydrogels are three-dimensional networks of hydrophilic polymers capable of absorbing large amounts of water or biological fluids without losing their structural integrity. This feature enables them to create microenvironments similar to soft tissues, thus promoting cell regeneration and favorable biological interactions (Nallusamy & Das, 2021).

From a functional perspective, chitosan hydrogels exhibit:

- High swelling capacity supports the transport of nutrients and the removal of cellular waste.
- Selective permeability is proper for gas exchange and controlled diffusion of biomolecules.
- Enzymatic biodegradability, due to the action of lysozyme in the human body, enables gradual integration with host tissue (Nguyen *et al*., 2023).
- Intrinsic antimicrobial properties, related to their positive surface charge, which helps prevent postoperative infections (Szymańska & Winnicka, 2015).

- Ease of modification, allowing for incorporating osteoinductive agents, nanoparticles, or progenitor cells through mild encapsulation techniques (Kim *et al*., 2023).

3.2.2 Role as 3D scaffolds: biocompatibility, porosity, and bioactivity

The design of three-dimensional (3D) scaffolds is an essential component of tissue engineering, as these structures must replicate the biochemical and structural environment of the bone matrix. Chitosan hydrogels fulfill several key requirements for this purpose (Figure 2):

- Biocompatibility: It is well documented that chitosan does not induce adverse immune responses under physiological conditions. Additionally, its cationic charge facilitates cell adhesion through interactions with ECM proteins and cell membranes (Freier *et al*., 2005).
- Porosity and interconnected architecture: The porous structure of hydrogels allows for efficient nutrient transport, metabolite removal, and cell migration—all critical factors in bone regeneration. For instance, pore sizes greater than 100 µm are optimal for vascularized bone tissue formation (Madihally & Matthew, 1999).
- Bioactivity: Through functionalization with peptides (e.g., RGD, BMP), hydroxyapatite nanoparticles (nHA), or growth factors, the hydrogel can be transformed into a bioactive system that not only supports but actively induces osteogenesis (Aguilar *et al*., 2019; Oliveira *et al*., 2021).
- Adaptable mechanical properties: While hydrogels alone do not exhibit the rigidity of cortical bone, they can be reinforced with bioceramics or inorganic phases to increase their elastic modulus without compromising biocompatibility (Kumar *et al*., 2019).

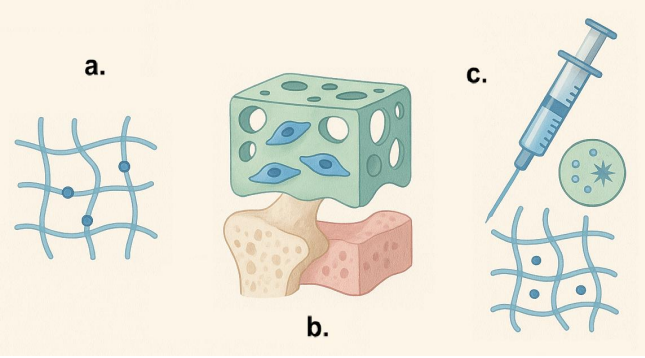

Figure 2. Hydrogels as bone tissue engineering platforms: a. functional characteristics of hydrogels; b. role as 3D scaffolds: biocompatibility, porosity, and bioactivity; c. injectable hydrogels, controlled release, and growth factors.

3.2.3 Injectable hydrogels, controlled release, and growth factors

One of the most promising innovations in this field is the development of injectable hydrogels, which can be applied to bone defects minimally invasively. These hydrogels can conform *in situ* to the shape of the defect and solidify via physical (thermogelation) or chemical (in situ crosslinking) mechanisms (Patois *et al*., 2009).

Furthermore, these systems can serve as controlled-release vehicles for bioactive agents. It has been demonstrated that growth factors such as:

- Bone morphogenetic proteins (BMP-2, BMP-7)
- Platelet-derived growth factors (PDGFs)
- Vascular endothelial growth factors (VEGFs)
- Transforming growth factors beta (TGF-β)

can be loaded into the hydrogel matrix and released in a sustained manner, thereby promoting not only osteogenesis but also angiogenesis, which is critical for the integration of newly formed tissue (Oliveira *et al*., 2021). These smart systems also allow the co-encapsulation of osteoprogenitor cells, leading to the development of multifunctional platforms capable of regenerating bone in complex environments with minimal surgical intervention (Li *et al*., 2023).

3.3 Origin of chitosan and extraction methods

Chitosan is a biopolymer derived from chitin, the second most abundant natural polysaccharide in the biosphere after cellulose. Chitin is found in a wide variety of invertebrates and microorganisms, and its conversion to chitosan through deacetylation processes is a critical step for biomedical applications. The origin of chitin directly influences the structural, physicochemical, and functional characteristics of the resulting chitosan. Therefore, it is essential to consider both the source and the extraction method when designing hydrogels for tissue engineering (Aguilar *et al*., 2019; Pellis *et al*., 2022).

3.3.1 Chitin sources: crustaceans, insects, fungi

a. Crustaceans: The most common commercial source of chitin is marine crustacean waste (shrimp, crabs, lobsters). This chitin is usually of the α-type, characterized by a highly crystalline and ordered structure, making it more resistant but also more difficult to deacetylate (Huq *et al*., 2022). It remains the most widely available option due to the large volume of waste generated by the fishing industry.
b. Insects: The exoskeletons of insects such as beetles, crickets, and larvae also contain chitin, generally with fewer minerals and allergens than crustaceans. Chitin extracted from insects typically presents a mix of α and β forms with a less compact structure, which may

facilitate its transformation into chitosan (Mohan *et al*., 2020; Hahn *et al*., 2020).

c. Fungi: Chitin from filamentous fungi and yeasts often exists as a chitin–glucan complex. It offers advantages such as being a more sustainable and low-allergen source and is independent of seasonality. However, its extraction is more complex, and the chitin content per unit of biomass is relatively low (Huq *et al*., 2022).

3.3.2 Source comparison

Table 2 summarizes the advantages, limitations, and degree of deacetylation (DD) for chitosan derived from the main biological sources:

3.3.3 Deacetylation processes: chemical, enzymatic, microwave, des

Transforming chitin into chitosan requires the removal of acetyl groups from N-acetylglucosamine units. This reaction can be performed using chemical, biological, or hybrid methods, each with different impacts on the degree of deacetylation (DD), molecular weight, and polymer chain distribution.

a. Chemical Method (alkaline and acidic): This is the most widely used industrial approach. It involves concentrated sodium hydroxide (NaOH) solutions at elevated temperatures (80–120 °C). It can achieve DD values above 80%, although it may degrade the polymer (Pellis *et al*., 2022). Pre-acidification with HCl is used for demineralization. Example: 50% NaOH at 100 °C for 1 hour (Pellis *et al*., 2022; Novikov *et al*., 2023).
b. Enzymatic Method: This approach uses chitinase and chitin deacetylase under mild conditions. While more environmentally friendly, it results in low DD values (10–30%) and slow kinetics, making it currently unfeasible for mass production (Harmsen *et al*., 2019).
c. Microwave-Assisted: This method relies on rapid, uniform heating of the sample in the presence of NaOH. It can accelerate the reaction within minutes, reaching up to 75% DD with reduced structural degradation (Tahir *et al*., 2024).
d. Steam Explosion: Involves high-pressure steam followed by rapid decompression to break down chitin structure, facilitating subsequent deacetylation. It is mainly used with shrimp and crab shell waste (Sugiyanti *et al*., 2019).
e. Deep Eutectic Solvents (DES): An emerging method using mixtures of hydrogen bond donors and acceptors (e.g., choline: malic acid) that melt at low temperatures. It enables controlled and environmentally friendly deacetylation. However, current efficiency remains limited (maximum ~40% DD in 24 hours) (Pellis *et al*., 2022).

3.4 Chemical and mechanical properties of chitosan

The performance of chitosan as a biomaterial for tissue engineering applications largely depends on its physicochemical properties. These characteristics determine its behavior in biological environments, its processability into forms such as hydrogels or films, and its compatibility with cells and tissues. The most relevant properties of chitosan for the design of hydrogels intended for bone regeneration are described below.

3.4.1 Chemical structure and functional groups

Chitosan is a linear polysaccharide composed of repeating units of D-glucosamine and N-acetyl-D-glucosamine, linked by β-(1→4) glycosidic bonds, similar to cellulose. Its structure results from the partial deacetylation of chitin (Figure 1). The degree of deacetylation (DD), typically ranging from 50% to 95%, defines the proportion of units with free amino groups (–NH_2), which are responsible for many of its functional properties (Aranaz *et al*., 2021; Ioelovich, 2014). Key functional groups include:

- Primary amino group (–NH_2) at carbon 2 of D-glucosamine → imparts positive charge in acidic media, enabling interactions with cell membranes and proteins.
- Hydroxyl groups (–OH) at carbons 3 and 6 → enable crosslinking and chemical modifications.
- Glycosidic bond (C1–O–C4) → provides structural integrity to the polymer chain.

These functional groups allow for crosslinking with aldehydes, organic acids, or ionic agents, and facilitate the incorporation of bioactive molecules, granting chitosan significant versatility in hydrogel formulation (Kim *et al*., 2023).

Table 2. Chitosan characteristics according to its source.

Source	Advantages	Limitations	Degree of Deacetylation (DD)
Crustaceans	- High availability - Established industrial methods - High chitin concentration	- Potential allergens - Presence of heavy metals - Use of harsh chemicals	56–98% (Triunfo *et al*., 2022)
Insects	- Low allergen content - High hydration and solubility capacity	- Requires melanin bleaching step - Limited large-scale production	62–98% (Mohan *et al*., 2020)
Fungi	- Animal protein-free source - More controlled process	- Difficult chitin–glucan separation - Lower yield -	70–93% (Huq *et al*.,

- Lower toxicity	Higher processing costs	2022)

3.4.2 pH, biodegradability, cell adhesion, and immunogenicity

a. pH and Solubility: Chitosan is soluble in acidic solutions (pH < 6.5) due to the protonation of its amino groups. It has a pKa of approximately 6.3, making it ideal for applications in slightly acidic biological environments such as wounds or inflammatory microenvironments. For applications in neutral pH settings (e.g., physiological bone tissue, pH ≈ 7.4), chemical modifications are required to enhance its solubility (Nguyen *et al*., 2023).
b. Biodegradability: Chitosan is degraded in the body by enzymes such as lysozyme, which cleaves the β-(1→4) glycosidic bonds. The degradation rate depends on the degree of deacetylation, molecular weight, and the extent of hydrogel crosslinking. This is advantageous for tissue engineering, as it allows the scaffold to be gradually resorbed as the regenerated tissue replaces it (Sawaguchi *et al*., 2015).
c. Cell Adhesion and Biointeractivity: Chitosan can promote cell adhesion due to electrostatic interactions with extracellular matrix glycoproteins and its compatibility with cellular receptors. The presence of amino groups enhances the adsorption of adhesion proteins (e.g., fibronectin, vitronectin), which in turn mediate the anchoring of osteoblasts and mesenchymal stem cells (Freier *et al*., 2005).
d. Immunogenicity: Numerous studies have shown that chitosan is biocompatible and non-immunogenic under normal conditions. However, it can modulate the immune response by activating antigen-presenting cells and promoting the production of cytokines that are beneficial for bone regeneration. Its effects on the Th1/Th2 axis have been explored as a potential adjuvant in immunotherapy (Li *et al*., 2021).

3.4.3 Mechanical properties

The mechanical properties of pure chitosan are relatively low compared to ceramic or metallic composites, but sufficient for soft tissue applications and, when reinforced, for bone tissue engineering.

The strength and stiffness of chitosan hydrogels can be modulated through:

- Polymer concentration
- Type of crosslinking (physical vs. chemical)
- Addition of nanofillers, such as hydroxyapatite or nanocellulose

Tensile Strength and Elastic Modulus:

- Chitosan films with 75–90% DD exhibit tensile strengths of 50–80 MPa (Chandra *et al*., 2023).
- Chitosan fibers show lower tensile strength, ranging from 11–20 MPa, depending on mechanical treatment and chain alignment.
- Pure chitosan hydrogels have an elastic modulus of approximately 0.29 ± 0.06 MPa, which can increase significantly with reinforcement using nano-hydroxyapatite (Kumar *et al*., 2019).

Porosity and Apparent Density: Structural porosity is critical for enabling cell migration and the formation of vascularized bone tissue. Chitosan hydrogels commonly exhibit porosity values greater than 90%, with pore diameters that can be tuned using techniques such as freeze-drying or electrospinning (Putri & Elsheikh, 2022).

Structural Flexibility: Zhang *et al.* (2021) demonstrated that chitosan hydrogels can exhibit sufficient flexibility to be knotted without breaking, while also supporting weights of up to 475 grams—highlighting their potential for use under moderate mechanical conditions (Zhang *et al*., 2021).

3.5 Chitosan hydrogel fabrication techniques

The biological performance of a hydrogel does not depend solely on the chemical composition of chitosan. The resulting microarchitecture from the fabrication process plays a crucial role in cell migration, nutrient diffusion, and integration with host bone. Below, the three most established processing strategies and their next-generation variants are described, highlighting how each technique modulates the scaffold's morphology, mechanics, and bioactivity.

3.5.1 Electrospinning: biomimetic nanofibers

Electrospinning generates fibers with diameters ranging from 50 nm to 2 µm that mimic the morphology of type I collagen, the main component of the bone matrix. Under a high-voltage electric field, the chitosan solution (often blended with PEO, PCL, or gelatin to adjust viscosity and conductivity) is stretched to form a Taylor cone and deposited as a nonwoven mesh on a collector (Subbiah *et al*., 2005).

Recent advances:

- Chitosan-hydroxyapatite blends have been shown to enhance mineralization and compressive strength of nanofibers, thanks to *in situ* apatite nucleation along the polymer-ceramic network (Purohit *et al*., 2024).
- The use of hydroxypropyl chitosan has enabled better wettability control and accelerated osteoblastic differentiation in *in vitro* models

(Wang *et al*., 2025a).
- Bibliometric studies report a >30% increase in publications on electrospinning-bone in the last two years, indicating rapid clinical adoption (Ma *et al*., 2025).

Mechanical and biological implications:

Electrospun mats exhibit moduli up to 10 MPa and submicron pores that support guided cell migration; being stackable, they enable porosity gradients for complex bone defects (Mazoochi & Jabbari, 2011; Saber *et al*., 2025). However, post-crosslinking (e.g., HCl vapors, genipin) is required to maintain integrity in aqueous environments.

3.5.2 Freeze-drying and freeze-casting: directional macroporosity

Freeze-drying starts with an acidic chitosan solution that is frozen and then subjected to sublimation under vacuum, leaving a spongy network with >90% porosity (Madihally & Matthew, 1999). Variants such as freeze-casting control the thermal gradient to orient ice crystals, generating axial channels that improve vascularization.

Recent advances

- Xylem-inspired structures with centripetal channels have doubled vascular infiltration rates in critical tibial defects (Wang *et al*., 2025b). Freeze-dried chitosan foams show significant bone regeneration in rat calvarial defects, comparable to autografts (Fathy *et al*., 2025).
- 2025 reviews highlight freeze-drying's relevance for achieving compressive strengths >0.5 MPa while maintaining interconnected porosity (Angraini *et al*., 2025).

Limitations and solutions:

The intrinsic fragility can be mitigated by incorporating β-tricalcium phosphate or bioactive glass nanoparticles, increasing the elastic modulus up to 1.2 MPa without compromising porosity (Putri & Elsheikh, 2022; Lourenço *et al*., 2024).

3.5.3 3D Printing and bioprinting: customization and geometric complexity

3D printing of chitosan pastes or inks allows the fabrication of anatomically precise scaffolds with defined porous architectures. Success depends on rheological adjustment: the ink must exhibit shear-thinning behavior and rapid gelation post-extrusion (Lazaridou *et al*., 2022).

Recent advances:

- Hybrid chitosan-bioactive glass inks have achieved resolutions of 200 µm and compressive strengths of 2 MPa, suitable for partial-load defects (Lourenço *et al*., 2024).
- 2024 reviews document the integration of microencapsulated growth factors, achieving controlled BMP-2 release for up to 28 days (Khan *et al*., 2024).
- Bibliometric analyses show that bioprinting with chitosan hydrogels is the fastest-growing research line (>25% annual growth) in bone regeneration (Zhang *et al*., 2025).

Clinical advantages:

- Customization: allows scaffold design based on patient CT/CBCT scans.
- Minimally invasive: thermoresponsive hydrogels can be printed *in situ* through endoscopic cannulas (Patois *et al*., 2009).
- Cell co-encapsulation: chitosan's moderate viscosity preserves >85% viability of MSCs during extrusion, supporting combined therapies.

Current challenges

- Reproducibility at industrial scale.
- Need for fast and cytocompatible crosslinking systems (e.g., visible light + riboflavin).
- Control of mineralization gradients to mimic the bone-cartilage interface.

3.6 Biomedical applications of chitosan hydrogels

The physicochemical versatility of chitosan and its hydrogels has enabled a wide range of biomedical applications, particularly in tissue engineering, targeted drug delivery, and wound healing. Below is a summary of the most relevant advances, emphasizing the relationship between "structure–process–property–function" from a comprehensive biomedical engineering perspective.

3.6.1 Osteochondral tissue engineering

Chitosan hydrogels (CHs) provide a hydrated three-dimensional microenvironment that supports the adhesion, proliferation, and differentiation of mesenchymal stem cells (MSCs), osteoblasts, and chondrocytes. This behavior is attributed to their cationic charge, which promotes the adsorption of extracellular matrix proteins (Freier *et al*., 2005; Kim *et al*., 2023).

- Osteogenesis *in vitro* and *in vivo*: CHs loaded with BMP-2 and hydroxyapatite nanoparticles have shown significant increases in the expression of ALP, Runx2, and osteocalcin, as well as lamellar

bone formation in critical tibial defects in rabbits (Oliveira *et al*., 2021; Li *et al*., 2023).

- Complex interfaces: Using multimaterial 3D bioprinting, CH/bioactive glass nanoparticle gradients have been created to replicate the osteochondral transition, achieving simultaneous integration of hyaline cartilage and subchondral bone in ovine models (Lazaridou *et al*., 2022).
- Neuro-osteogenesis: Recent studies describe CHs doped with gallium ions capable of simultaneously stimulating bone regeneration and peripheral reinnervation, demonstrating the potential for bifunctional platforms (De León-Oliva *et al*., 2023).

3.6.2 Controlled drug delivery systems

The polycationic nature of chitosan (Table 3) enables the formation of ionic complexes with anionic molecules and modulation of release kinetics via pH or temperature changes (Nguyen *et al*., 2023).

These formulations have been explored for both local chemotherapy—avoiding systemic toxicity—and sustained antibiotic delivery in bone grafts, reducing recurrence of osteomyelitis (Aguilar *et al*., 2019).

3.6.3 Wound healing

Although the main focus of this article is bone regeneration, it is worth noting that CHs were initially recognized for their wound healing potential. Their hemostatic capacity, antimicrobial activity, and angiogenesis stimulation accelerate the classical four phases of tissue repair (Azad *et al*., 2004; Ansari, 2019). Self-healing hydrogels made from oxidized chitosan and gelatin achieved complete closure of diabetic wounds in 10 days, compared to 16 days in standard gauze-treated controls (Li *et al*., 2023).

Table 3. Stimulus-responsive mechanisms for drug release with chitosan.

Strategy	Control Principle	Examples and Results
Thermosensitive (CS-β-GP)	Gelation at 37 °C	Sustained doxycycline release for 14 days, reducing peri-implant inflammation (Patois *et al*., 2009).
pH-responsive	$–NH_3^+$ deprotonation (pH > 6.5)	CS/alg–Ca^{2+} microgels with accelerated release in acidic necrotic regions (Sangnim *et al*., 2023).
Redox-sensitive	Disulfide bridges with cystamine	Pulsatile paclitaxel delivery in the presence of tumor GSH (Yanat & Schroën, 2021).

3.7 Current challenges and future perspectives

Despite significant progress in the development of chitosan hydrogels (CHs) for biomedical applications, structural, technological, and regulatory challenges remain that hinder their large-scale clinical translation. The following discussion outlines the main technical obstacles in the field, as well as scientific projections toward a new generation of bioactive scaffolds and personalized regenerative therapies.

3.7.1 Limited solubility of chitosan

One of the main challenges in handling CHs is their low solubility in neutral or basic media, due to the pH-dependent protonation of chitosan's primary amine groups ($–NH_2$). This property restricts their direct application in physiological environments and necessitates the use of weak acids (such as acetic, lactic, or citric acid) for initial dissolution (Aranaz *et al*., 2021; Huq *et al*., 2022).

To address this issue, several strategies have been developed:

- Chemical modifications: Quaternization and sulfonation improve solubility at neutral pH, enhancing the polymer's dispersion in biological fluids (Li *et al*., 2021).
- Formulation with soluble polymers: Combinations with PEG, PVA, or hyaluronic acid increase the miscibility of CHs in physiological solutions without compromising biofunctionality (Nguyen *et al*., 2023).
- Use of green solvents and deep eutectic systems (DES): Recent technologies have demonstrated the effectiveness of solvents like choline–oxalic acid for dissolving chitin and obtaining safer CHs (Pellis *et al*., 2022; Tahir *et al*., 2024).

3.7.2 Need for standardization of degree of deacetylation (DD)

The degree of deacetylation (DD) is one of the most critical structural parameters influencing chitosan's bioactivity and degradability. A high DD (>85%) promotes cell adhesion and interaction with negative charges, while a lower DD (<70%) enables enhanced enzymatic degradation (Freier *et al*., 2005; Ioelovich, 2014). However, the lack of standardized methods for its measurement and reporting hinders reproducibility across studies.

Current efforts include:

- Advanced metrology: Use of NMR spectroscopy, quantitative FTIR, and chromatography to reliably characterize DD (Novikov *et al*., 2023).
- Harmonized international protocols: Regulatory agencies (FDA, EMA) now require precise

specifications of DD and molecular weight in medical applications, promoting polymer traceability from source to final product (Mohan *et al*., 2020; Hahn *et al*., 2020).
- Development of CH databases: Libraries of well-characterized chitosans are being compiled to facilitate their selection according to the intended application (De Alvarenga, 2011; Aranaz *et al*., 2021).

3.7.3 Personalized bioprinting and advanced therapies

With the rise of personalized medicine, CHs are emerging as key bioinks in 3D bioprinting, where their viscoelasticity, biocompatibility, and water retention capacity enable the creation of anatomically precise structures for bone, cartilage, and vascular regeneration (Lazaridou *et al*., 2022; Kim *et al*., 2023).

Ongoing challenges:

- Precise tuning of bioink rheology to maintain structural fidelity and post-printing cell viability.
- Integration with active biomolecules (e.g., growth factors, messenger RNA) to enable spatiotemporally controlled release.
- Multimaterial fabrication: Combination with ceramics such as β-TCP or conductive polymers to generate osteoinductive and even electrostimulative environments.

Future perspectives:

- CHs functionalized with specific ligands will enable the development of smart scaffolds capable of responding to bone microenvironment stimuli such as pH or reactive oxygen species (Zhang *et al*., 2021).
- Combined HQ–stem cell–CRISPR therapies could transform the treatment of congenital or degenerative bone diseases (Rondón *et al*., 2023; De León-Oliva *et al*., 2023).

4 Conclusion

From a biomedical-engineering standpoint, chitosan is firmly established as a natural polymer with high potential for developing hydrogels aimed at regenerative therapies. Its structural versatility, biocompatibility, gelling ability, and tunable physicochemical properties make it a multifaceted platform for advanced medical treatments—especially for bone-tissue regeneration.

Recap of chitosan's benefits in hydrogels

Chitosan hydrogels (CHs) possess a highly hydrated, porous architecture that mimics the bone extracellular microenvironment, promoting cell adhesion, nutrient transport, and osteoblastic proliferation. Key advantages include:

- Controlled biodegradability, adjustable through structural modifications of the polymer.
- Chemical or physical cross-linking capability, enabling injectable, self-supporting, or thermally stable systems.
- High affinity for biomolecules such as morphogenetic proteins (BMPs), antibiotics, and growth factors.

These features position CHs as optimal vehicles for targeted drug delivery, 3-D scaffolds for tissue engineering, and smart systems responsive to physiological stimuli.

Importance of origin and synthesis method

A critical determinant of CH performance is the chitosan's origin and extraction technique. Alternative sources—such as insects and fungi—and modern methods like microwave-assisted deacetylation yield chitosans with more consistent, sustainable molecular profiles suited to biomedical use.

Equally essential is standardizing the degree of deacetylation and molecular weight to ensure material reproducibility and safety in clinical settings. This underscores the need for engineering approaches that integrate chemical-process control, advanced biomaterial characterization, and preclinical functional validation.

Potential applications in bone regeneration and beyond

CHs have shown promising results in repairing critical bone defects, acting as bioactive scaffolds that not only provide mechanical support but also enhance mineralization and new bone formation. Functionalization with nanocomposites or bioactive factors broadens their application to:

- Cartilage and skin regeneration.
- Sustained delivery of anti-inflammatories or antibiotics for osteo-articular lesions.
- 3-D bioprinting of patient-specific tissues by integrating stem cells and modular bioinks.

Consequently, chitosan hydrogels stand out as a strategic technology in modern regenerative medicine, where biomedical engineering supplies rational design tools, advanced manufacturing, and comprehensive *in vitro*/*in vivo* evaluation to accelerate the transition toward effective, safe, and accessible clinical therapies.

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