Journal of Rare Cardiovascular Diseases
ISSN: 2299-3711 (Print) | e-ISSN: 2300-5505 (Online)
www.jrcd.eu
RESEARCH ARTICLE

CHITOSAN DERIVATIVES AS TRANSFORMATIVE NATURE BIOMATERIAL IN REGENERATIVE MEDICINE AND TISSUE ENGINEERING

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Article History
Received: 10.07.2025
Revised: 14.07.2025
Accepted: 05.08.2025
Published: 08.09.2025

Abstract: Chitosan, a naturally deacetylated polysaccharide derived from chitin, represents a versatile biopolymer for regenerative mtissue engineering applications due to its biocompatibility, enzymatic biodegradability, and compositional analogy to extracellular matrix glycosaminoglycans. This systematic literature review synthesizes contemporary evidence regarding chitosan's physicochemica attributes, structural modifications, and therapeutic applications with emphasis on translational feasibility for clinical tissue regeneration. Chitosan's molecular weight and degree of deacetylation are tunable parameters enabling chemical derivatization, enzymatic modification. and physical processing to yield biomaterials with optimized mechanical and biological functionality. Carboxymethylation and enzymatic hydrolysis produce chitosan oligosaccharides and derivatives demonstrating enhanced aqueous solubility and bioavailability, while composite formulations incorporating polymeric matrices or bioactive mineral phases enhance osteoconductivity and load-bearing capacity. Pharmacokinetic investigations establish favorable absorption kinetics and biodegradation profiles, with hepatic and renal eliminatior mediating conversion to non-immunogenic metabolites. Chitosan-functionalized scaffolds facilitate wound healing via thrombogenic mechanisms and fibroblast proliferation with collagen synthesis, whereas multifunctional biocomposites promote chondrogenesis osteogenesis, and intervertebral disc repair. Emerging evidence demonstrates efficacy in vascular tissue engineering, post-myocardia infarction therapeutic delivery, and endothelial regeneration through modulation of inflammatory responses and prevention of restenotic pathology. Limitations include accelerated enzymatic degradation kinetics, diminished mechanical retention in vivo, and suboptimal tissue integration. Prioritized research directions encompass development of structurally optimized scaffolds with regulated degradation kinetics incorporation of bioactive molecules and progenitor cells, and implementation of advanced biofabrication methodologies including electrospinning and additive manufacturing. Large-scale animal model validation and controlled clinical trials are prerequisite for translational implementation Collectivelv chitosan and its derivatives constitute promising candidates.

Keywords: Chitosan; Biopolymer; Tissue engineering; Wound healing; Biomedical applications.

INTRODUCTION

Chitosan, a deacetylated derivative of chitin, is among the most favored natural polymers in tissue engineering alongside gelatin and collagen due to its low immunogenicity, natural degradability, biocompatibility, and structural similarity to the extracellular matrix (ECM) (Ferreira et al., 2023; dos Santos et al., 2006; Gomes et al., 2017; Khalaf et al., 2023; Khalilimofrad et al., 2023) Over recent decades, chitosan has been developed for biomedical applications and extensively applied across wastewater treatment and agriculture (Atheena et al., 2024; Bruckmann et al., 2025; Savary et al., 2024), paper manufacturing and packaging (Niu et al., 2024), food additives and preservatives, biodegradable food packaging (Jin et al., 2024; Flórez et al., 2022), textiles (Elamri et al., 2023; Costa et al., 2022), cosmetics (Kulka et al., 2023; Guzmán et al., 2022), metal reduction or chelating materials, and civil engineering (Joseph et al., 2021; Rukhsar et al., 2025). Its excellent biocompatibility, favorable biodegradability, and low toxicity make it particularly suitable for biomedical use, with its polycationic backbone enabling chemical modification to produce derivatives, composites, and nanomaterials with unique properties (Jayakumar et al., 2010; Liaqat & Eltem, 2018, Žigrayová et al., 2024; Manna et al., 2023). Substitution of reactive amino groups through crosslinking, grafting, and copolymerization affects acid-base behavior, electrostatic characteristics, biodegradability, solubility, and metal ion chelation, enabling broad application in pharmaceuticals and biomedical innovations (dos Santos et al., 2006; Nicolle et al.,2021; Žigrayová et al., 2024; Desai et al. 2023). Its cationic nature facilitates electrostatic interaction with negatively charged biomolecules for gene delivery, cell culture systems, and tissue engineering scaffolds, while its derivatives possess antimicrobial, antioxidant, anti-inflammatory, analgesic, immunomodulatory, anticancer, antidiabetic, and hemostatic effects (Yadav et al., 2023; Mawazi et al., 2024; Aranaz et al., 2021; Francesko & Tzanov, 2010; Xia et al., 2011).

NATURE OF CHITOSAN BIOMATERIAL

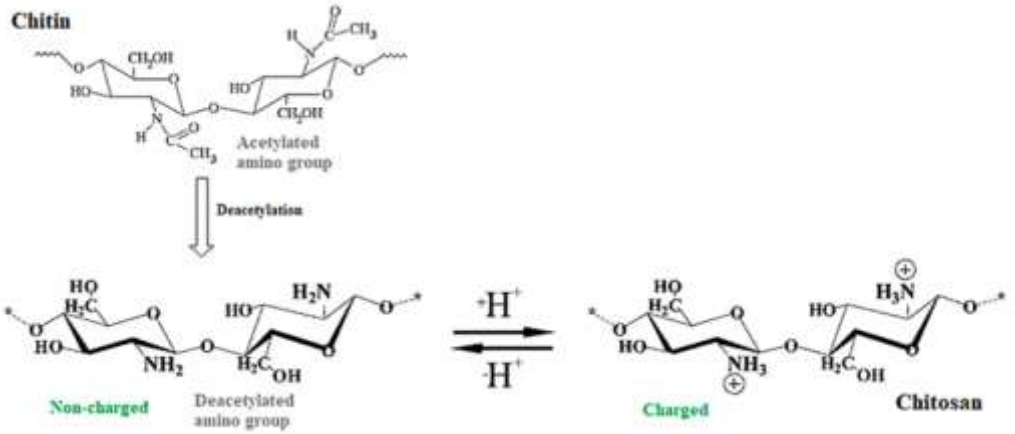
Chitosan is a natural biomaterial obtained from chitin, a structural polysaccharide abundant in crustacean exoskeletons, squid pens, insect cuticles, and fungal cell walls (Singh et al., 2017; Islam et al., 2022; Santos et al., 2020; Piekarska et al., 2023; Pellis et al., 2022; Crognale et al., 2022). Chitin is a hard, nitrogenous, white

polysaccharide—the second most abundant natural biopolymer after cellulose—composed of β-(1→4)-linked N-acetylglucosamine units, existing in three polymorphic forms (α, β, γ), with natural turnover facilitated by bacterial enzymes (Guan et al., 2022; Elieh-Ali-Komi & Hamblin, 2016; Piekarska et al., 2023; Santos et al., 2020).

Chitosan is produced by partial deacetylation of chitin to expose –NH_2 groups, with the degree of acetylation (DA) distinguishing chitin (highly acetylated) from chitosan (<50% acetyl content) (Elieh-Ali-Komi & Hamblin, 2016; Piekarska et al., 2023; Pellis et al., 2022; Guan et al., 2022). Conventional production uses concentrated NaOH or KOH under strong alkaline conditions, though this generates significant chemical waste, while microwave-assisted deacetylation achieves 73.86% deacetylation in 60 minutes versus 180 minutes for conventional methods (Pratiwi et al., 2023, El Knidri et al., 2019; Cheng et al., 2020). Enzymatic methods utilizing chitinases and chitin deacetylases enable eco-friendly production with fine control over molecular weight and degree of deacetylation, producing well-defined chitosan and chitooligosaccharides (COS) (Kaczmarek et al., 2019; Gonçalves et al., 2021). Physical degradation methods such as ultrasound, microwave, and radiation enable rapid depolymerization of chitosan by cavitation and shear forces, reducing molecular weight while preserving functional groups and enhancing solubility (Pandit et al., 2021; Vallejo-Domínguez et al., 2020; Cheng et al., 2020). Among available approaches, enzymatic and microwave-assisted enzymatic hydrolysis are regarded as the most desirable due to their ability to produce high-yield chitosan hydrolysates rich in bioactive glucosamine and oligosaccharides, with superior environmental and functional advantages (Pellis et al., 2022; Ewais et al., 2025).

STRUCTURAL AND PHYSICOCHEMICAL PROPERTIES

Chitosan is a biodegradable linear polysaccharide composed of β-(1→4)-linked D-glucosamine and N-acetyl-D-glucosamine units, with degree of deacetylation determining crystallinity, solubility, and reactivity, enabling transformation into powders, films, fibers, beads, and hydrogels for biomedical applications (Rkhaila et al., 2021, Gzyra-Jagieła et al., 2019, Khalaf et al., 2023, Edo et al., 2025). Molecular weight and degree of deacetylation are key determinants of chitosan solubility, viscosity, and mechanical strength. Chitosan is inherently insoluble in water but becomes soluble in dilute organic acids such as acetic, formic, lactic, citric, and ascorbic acids under acidic conditions (pKa ≈ 6.5), where protonation of amino groups enhances its solubility and processability (**Echazú** et al., 2016; Roy et al., 2017; Sogias et al., 2010; Aranaz et al., 2021). Its polycationic nature enables electrostatic interaction with negatively charged biomolecules (nucleic acids, proteins, drugs) facilitating complex formation, mucoadhesion, controlled release, and cellular uptake for biomedical applications (de Sousa Victor et al., 2020; Mohammadi et al., 2021).



Despite its excellent biocompatibility, chitosan's practical utility is limited by poor solubility under neutral and alkaline conditions, instability at physiological pH, and variability in molecular weight and degree of deacetylation, which negatively affect reproducibility (Szymańska & Winnicka 2015; Kołodziejska et al. 2021). Chemical, physical, and enzymatic modifications — including acylation, carboxylation, alkylation, quaternization, and graft copolymerization — enhance the solubility, thermal stability, rheological behavior, and biocompatibility of chitosan (Wang W et al. 2020; Suryani et al. 2024). Reducing molecular weight decreases hydrogen bonding, resulting in a less compact polymeric network with greater chain mobility, which significantly enhances solubility and makes low-molecular-weight chitosan (LMW-chitosan) and chitooligosaccharides (COS) more suitable for biomedical applications (Guan et al., 2019; Gonçalves et al., 2021; Gzyra-Jagieła et al., 2019).

BIODEGRADATION OF CHITOSAN

Chitosan undergoes enzymatic degradation by lysozyme, chitinases, and bacterial enzymes, as well as non-specific degradation by α-amylases and lipases, with the degradation rate influenced by the degree of deacetylation, molecular weight, and crystallinity (Yadav et al., 2019; Schmitz et al., 2019; Liang et al., 2018; Rkhaila et al., 2021). Chitosan is broken down into absorbable monosaccharides and non-toxic low-molecular-weight chitosan derivatives and COS, supporting safe biomedical applications. These degradation products are particularly suitable for composite scaffolds, demonstrating controlled biodegradation and biocompatibility for bone tissue engineering (Kaczmarek-Szczepańska et al., 2025; Pandit et al., 2021; Khan et al., 2024; Yodsanga et al., 2025).

CLINICAL AND PHARMACEUTICAL PERSPECTIVES

Chitosan exhibits distinct pharmacokinetic behavior depending on its molecular structure, solubility, and route of administration. Its cationic and mucoadhesive nature enables strong interaction with negatively charged mucins and epithelial membranes, which prolongs gastrointestinal residence time and enhances drug absorption by transiently opening epithelial tight junctions (Alfatama et al., 2024; Hussain et al., 2025; Azman et

al., 2022; Wongwanakul et al., 2023). Upon oral administration, chitosan is partially absorbed through paracellular and endocytic pathways. Low-molecular-weight COS (~3.8 kDa) demonstrate more than 20-fold greater intestinal permeability compared with high-molecular-weight chitosan (~230 kDa). COS absorption occurs predominantly in the small intestine via carrier-mediated transport and passive diffusion, followed by systemic distribution to the liver, kidney, and spleen (Chae et al., 2005; Guan & Feng, 2022; Kean & Thanou, 2010; Naveed et al., 2019). Unabsorbed COS reach the distal intestine, where they undergo microbial fermentation, while higher-molecular-weight chitosan (~300 kDa) is subjected to enzymatic degradation into fragments smaller than 45 kDa. Hepatic tissues play a dominant role in the biodegradation of high-molecular-weight chitosan (>200 kDa), generating fragments ranging from 1–50 kDa (Dong et al., 2010; Shao et al., 2015). Renal elimination contributes significantly to the excretion of COS, and clinical studies indicate that chitosan is well tolerated, with oral doses up to 4.5 g/day administered for 12 weeks producing no significant adverse effects. Furthermore, fluorescein-labeled carboxymethyl chitosan demonstrates rapid absorption and approximately 85% urinary excretion within 11 days (Naveed et al., 2019; Markovsky et al., 2012; Hamedi et al., 2018; Dong et al., 2010).

Chitosan-based nanoparticles enhance intestinal permeability and protect encapsulated drugs from enzymatic degradation and acidic hydrolysis, thereby enabling sustained release. Electrostatic complexes improve mucus penetration, reduce premature clearance (Dube et al., 2010; Xia et al., 2022; Alfatama et al., 2024; Hussain et al., 2025; Sethi et al., 2021), and support enhanced oral and targeted delivery of insulin, catechins, and peptide-based therapeutics (Aqib et al., 2025; Fatahi et al., 2025). Water-soluble chitosan derivatives exhibit rapid absorption and extensive biodegradation, whereas water-insoluble high-molecular-weight chitosan undergoes slower enzymatic degradation with prolonged tissue persistence (Dong et al., 2010; Li et al., 2015; Kołodziejska et al., 2021). Hepatic and renal pathways play dominant roles in biodegradation, with lysozyme-mediated cleavage constituting a key enzymatic route in plasma, liver, kidney, and urine. Additionally, chitosan–genipin hydrogels undergo both hydrolytic and enzymatic degradation, with nanoparticles eliminated via biliary and urinary excretion and demonstrating minimal cytotoxicity (Onishi & Machida, 1999; Li et al., 2015; Shao et al., 2015; Jiang et al., 2019; Reay et al., 2022; Khan et al., 2024; Sonin et al., 2020).

COS exhibit route-dependent pharmacokinetics, with their smaller molecular size and increased solubility enabling superior epithelial and mucosal barrier permeability through paracellular transport and endocytic pathways, thereby facilitating systemic bioavailability even at low concentrations (Gonçalves et al., 2021; Liu et al., 2021; Lee et al., 2025). In vivo studies have demonstrated that COS are absorbed through the intestinal mucosa and systemically distributed to the liver and kidneys for metabolism and clearance without bioaccumulation or organ damage, with high bioavailability following oral administration and slower biodegradation after parenteral administration, indicating route-dependent clearance (Yu et al., 2021; Guan & Feng, 2022; Gonçalves et al., 2021). Their small molecular size facilitates rapid renal excretion, while enzymatic degradation ensures complete elimination, and toxicological evaluations confirm their biocompatibility, showing no cytotoxic or mutagenic effects at physiological concentrations (Phil et al., 2019; Roman et al., 2019; Xia et al., 2011; Chen et al., 2022; Guan & Feng, 2022).

CHITOSAN DERIVATIVES MODIFICATIONS AND BIOMEDICAL APPLICATIONS

Chitosan's physicochemical limitations—such as low water solubility, high viscosity, and limited mechanical strength—can be addressed through chemical modifications, including acylation, phosphorylation, carboxylation, alkylation, quaternization, and graft copolymerization, targeting functional groups such as amino, hydroxyl, and acetamido moieties. These modifications enhance solubility, thermal stability, rheological properties, antioxidant capacity, and antimicrobial activity (Mohan et al., 2020; Guan & Feng, 2022; Žigrayová et al., 2024; Nicolle et al., 2021; Echazú et al., 2016).

Carboxymethyl chitosan (CMC) maintains remarkable solubility under neutral and alkaline pH, overcoming the limitations of native chitosan, with improved antibacterial efficacy and favorable biocompatibility. Its amphoteric character allows ionization across physiological pH ranges (Cao & He, 2025; Yadav et al., 2023; Dong et al., 2010; Rajoka et al., 2020). Carboxymethyl chitosan (CMC) possesses abundant amino and carboxyl groups that interact with inorganic ions, forming hydrogels with beneficial wound healing effects, including reduced inflammatory infiltration, increased collagen formation, and enhanced epidermal regeneration comparable to commercial alternatives. These hydrogels also show potential for targeted liver drug delivery (Li et al., 2024; Kłosiński et al., 2023; Chen et al., 2022; Fatullayeva et al., 2022).

Chitosan-based composites incorporating polymers (polyethylene glycol, polylactic acid, collagen, starch) and inorganic materials (bioactive glass, ceramics) have been extensively explored for drug delivery and tissue engineering, enhancing biocompatibility, mechanical integrity, drug-loading efficiency, and cellular interactions (Fatullayeva et al., 2022; Chen et al., 2022; Carvalho et al., 2024). Chitosan oligosaccharides (COS)—low-molecular-weight derivatives consisting of 2–10 glucosamine units with molecular weights <5 kDa—demonstrate dramatically enhanced water solubility due to disrupted hydrogen bonding, yielding a more open and hydrophilic structure with antioxidant, anti-inflammatory, and immunomodulatory effects, supporting applications in oral delivery, wound healing, diabetic wound healing, and dermatological applications for skin barrier protection and photoaging prevention (Gonçalves et al., 2021; Guan et al., 2019; Guan & Feng, 2022; Li et al., 2025; Lee et al., 2025; Wang et al., 2022).

Chitosan is processed into diverse formats—hydrogels, films, scaffolds, nanoparticles, nanofibers, sponges, and microspheres—each optimized for specific applications. Hydrogels provide high water retention and controlled drug release for wound healing and tissue regeneration; films serve as bioactive dressings supporting cell attachment and gas exchange; porous scaffolds mimic the extracellular matrix for cell adhesion and tissue integration; and nanoparticles enhance drug encapsulation and targeted delivery (Shah et al., 2025; Yadav et al., 2024; Rajinikanth et al., 2024; Chicea & Nicolae-Maranciuc, 2024; Gonciarz et al., 2025; Kravanja et al., 2019; Abourehab et al., 2022; Zhao et al., 2018). Chitosan-based composites overcome the limitations of pure chitosan by blending with natural polymers (gelatin, collagen) to enhance biocompatibility and cellular adhesion for soft tissue engineering, while incorporation of inorganic components (silica, hydroxyapatite) produces hybrid scaffolds with improved porosity, structural integrity, and osteoinductive

potential, promoting bone tissue formation and mineralization (Khalilimofrad et al. 2023; Brebu et al. 2024; Lee et al. 2009; Pérez-Moreno et al. 2023).

WOUND HEALING AND REGENERATIVE MEDICINE

Chitosan-based formulations demonstrate remarkable versatility in wound healing and tissue engineering due to their biocompatibility, biodegradability, and ability to support cellular activities, with the cationic nature enabling electrostatic interactions with negatively charged cell membranes to enhance fibroblast adhesion, proliferation, and collagen synthesis, while hydrogels facilitate hemostasis, promote angiogenesis, and provide controlled drug release for applications in skin, bone, and cartilage engineering (Rajinikanth et al. 2024; Shah et al. 2025; Abourehab et al. 2022; Gonciarz et al. 2025; Yadav et al. 2023; Kravanja et al. 2019). Chitosan exhibits potent hemostatic activity through erythrocyte aggregation, platelet activation, and fibrin network formation, accelerating blood clotting even under anticoagulated conditions. Its cationic amino groups enable rapid adhesion and clot stabilization, while chitosan-based dressings reduce bleeding time and promote tissue regeneration through macrophage-mediated healing and fibroblast proliferation. Recent evidence demonstrates macrophage polarization toward the M2 phenotype, reducing inflammation and scar formation (Zhang S et al., 2024; Fan et al., 2023; Gheorghiță et al., 2023; Pogorielov & Sikora, 2015; Periayah et al., 2012; Xia et al., 2022; Cassano et al., 2024; Zhang W et al., 2024).

Chitosan-based hydrogels accelerate wound healing through hemostasis, anti-inflammation, cell proliferation, and tissue remodeling. Their positively charged amino groups enhance fibroblast adhesion, migration, and collagen deposition, while the porous, moist structure supports oxygen exchange and exudate absorption. Intrinsic antibacterial activity and delivery of bioactive agents reduce infection and promote angiogenesis. The combination of biodegradability, biocompatibility, and self-healing capacity further enhances clinical applicability (Che et al., 2024; Liu et al., 2018; Rossary et al., 2025; Wang X et al., 2023; Zhang X et al., 2024).

CHITOSAN-BASED MATERIALS IN TISSUE ENGINEERING

Cartilage Tissue Engineering

Articular cartilage, an avascular tissue composed of chondrocytes embedded in an extracellular matrix (ECM) rich in collagen and glycosaminoglycans (GAGs), provides load-bearing and shock-absorbing functions but has limited intrinsic repair capacity, with injuries exceeding a few millimeters rarely healing spontaneously (Sophia Fox, 2009; Pueyo Moliner, 2025). Chitosan, structurally analogous to GAGs, electrostatically interacts with negatively charged ECM components to promote chondrocyte proliferation and matrix synthesis. Blends of chitosan with natural polymers such as gelatin, collagen, alginate, and silk fibroin demonstrate favorable properties for cartilage regeneration, including chitosan-gelatin hydrogels exhibiting high mechanical strength (Young's modulus 3.25 MPa; compressive strength 2.15 MPa), suitable degradation rates, and strong cell adhesion (Mukhtar, 2021; Stefanache, 2025; Oprenyeszk, 2015; Sivanesan, 2022; Shen, 2015).

Recent modifications improving solubility and bioactivity include carboxymethyl chitosan-silk fibroin composites, which support chondrocyte proliferation and glycosaminoglycan synthesis both in vitro and in vivo (Sivanesan, 2022); chitosan-hyaluronic acid and chitosan-chondroitin sulfate hydrogels, which improve chondrocyte phenotype maintenance and extracellular matrix production (Hamidi, 2025); chitosan-glucose derivative membranes, which enhance cartilage regeneration in rabbit models by promoting tissue integration and matrix formation (Verma, 2024); and chitosan oligosaccharides delivered via extracellular vesicles from mesenchymal stem cells, which accelerate cartilage repair and reduce osteoarthritic changes in vivo (Chuang, 2024; Li, 2021).

Bone Tissue Engineering

Bone tissue engineering designs three-dimensional (3D) scaffolds replicating the extracellular matrix with high biocompatibility, interconnected porosity, osteoconductivity, and sufficient mechanical strength to support cell adhesion, proliferation, and differentiation. Chitosan and its low-molecular-weight derivatives (COS, CMC) are natural polysaccharides with modifiable amino and hydroxyl groups, enabling enhanced solubility, adhesion, and mechanical performance (He et al., 2025; Zhang et al., 2025; Jafernik et al., 2023). Chitosan's cationic nature facilitates electrostatic interactions with negatively charged biomolecules, improving its function as a structural and bioactive matrix, while COS exhibits higher solubility and reactivity suitable for hydrogels and bioadhesives, and CMC scaffolds display tunable porosity, antibacterial activity, and osteogenic potential (Jafernik et al., 2023; Jafari et al., 2020; Zhou et al., 2024). However, native chitosan exhibits relatively weak mechanical properties and lacks inherent osteoconductivity compared to natural bone, limiting its standalone performance (Pellis et al., 2022; Jafernik et al., 2023).

Chitosan-based composites incorporating ceramics, biopolymers, or inorganic nanoparticles enhance structural integrity and biological performance, with bioactive ceramics (hydroxyapatite, calcium phosphate, carbonate apatite) improving mechanical strength and osteoconductivity, thereby promoting new bone formation both in vitro and in vivo (Zhou et al., 2024; Ressler, 2022; Li et al., 2025; Bharathi et al., 2022; Saravanan et al., 2016; Ariani, 2023; Li et al., 2023). Hybrid scaffolds combining chitosan with natural (gelatin, collagen, alginate) or synthetic polymers (PLA, PCL, PEG) improve structural integrity and mimic the extracellular matrix, facilitating osteogenic differentiation and vascularization, with strontium- and zinc-doped hydroxyapatite-chitosan composites enhancing alkaline phosphatase activity and mineral deposition (Rodríguez-Vázquez et al., 2015; Oryan and Sahvieh, 2017; Kołodziejska et al., 2021; Rajoka et al., 2020; Li et al., 2023).Injectable thermosensitive chitosan-gelatin or chitosan-bioactive glass hydrogels demonstrate favorable gelation behavior, biocompatibility, and degradation kinetics supporting osteogenic differentiation and bone defect repair, with incorporation of inorganic phases (hydroxyapatite, metal-organic frameworks) enhancing osteoconductivity and bioactivity, while recent multifunctional injectable hydrogels simultaneously promote osteogenesis, angiogenesis, and immunomodulation (Tian et al., 2022; Pellis et al., 2022; Li et al., 2025; He et al., 2025; Zhang et al., 2025; Zhou et al., 2024).

Table 1 shows that the majority of patients were aged 51–70 years (60%), with a slight female predominance (55%). Overweight and obesity were highly prevalent (66%). Treatment compliance at the time of inclusion to

present study was poor. Among the patient prescribed treatment for hypertension, only 3.2% were compliant with the therapy. Vitamin D deficiency was widespread (62%). Echocardiography revealed high rates of structural heart changes: PWD was abnormal in 92%, IVST in 90%, LVMI in 66%, and EDD in 53%. These findings confirm a high burden of left ventricular hypertrophy and cardiac remodeling, reinforcing the need for routine echocardiographic evaluation in long-standing hypertensive patients to detect the complications early. (figure 1)

Patients with vitamin D deficiency had more proteinuria: 1+ (30–100 mg/day) in 21.0%, 2+ (100–300 mg/day) in 4.8%, and 3+ (>300 mg/day) in 11.3%.

In contrast, insufficiency showed only 1+ in 5.0% and 2+ in 5.0%, while sufficiency showed 2+ in 11.1% with no 1+ or 3+ cases. Negative proteinuria was most common in insufficiency (90.0%) and sufficiency (88.9%) compared to deficiency (62.9%). The association was statistically significant (χ^2 = 13.354, df = 6, **p = 0.038**).(table 2)

Abnormal LVMI was common in all groups, seen in 71.0% of vitamin D–deficient, 60.0% of insufficient, and 55.6% of sufficient patients, though the difference was not significant. Abnormal IVST was also frequent, occurring in 93.5% of deficient, 85.0% of insufficient, and 83.3% of sufficient cases, with no significant difference(Figure 2)

For PWD, abnormalities were most frequent in deficient cases (96.8%) and least in sufficient children (77.8%), showing a significant association with vitamin D status (p = 0.031).

Abnormal EDD was found in 59.7% of deficient, 35.0% of insufficient, and 50.0% of sufficient cases, but this was not statistically significant.

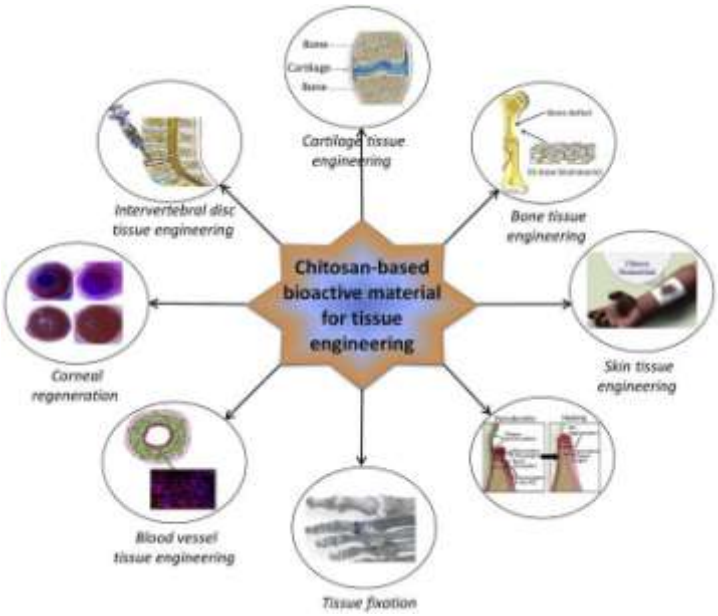


Fig. 2. Schematic representation of applications of chitosan-based bioactive materials.[42]

DISCUSSION

Intervertebral Disc Tissue Engineering

The intervertebral disc (IVD), composed of the nucleus pulposus (NP), annulus fibrosus (AF), and vertebral endplates (VEPs), functions as the primary load-bearing structure but is largely avascular, limiting its intrinsic regenerative capacity. Degeneration is characterized by proteoglycan depletion, extracellular matrix disorganization, and disc height loss, while conventional surgical treatments often fail to restore native disc biomechanics (Stergar 2019; Choi 2019; Nie 2015; Di Martino 2005). Chitosan, structurally similar to glycosaminoglycans, exhibits biodegradability, cytocompatibility, and hemostatic properties suitable for intervertebral disc regeneration, with chitosan-based gels supporting cell viability and matrix deposition, and injectable chitosan–cellulose nanofiber hydrogels demonstrating enhanced viscoelasticity and mechanical

stability in porcine spine models (Rodríguez-Vázquez 2015; Roughley 2006; Doench 2018, 2019; Guo 2025).

Hybrid hydrogels combining decellularized NP matrix, chitosan, and GDF5-loaded microspheres promote ECM synthesis and delay disc degeneration in vivo, with chitosan–gelatin hydrogels supplemented with link-N peptides enhancing ECM deposition and promoting proteoglycan and collagen synthesis, while chitosan–gelatin composites provide mechanical support and biocompatibility enabling in situ regeneration of intervertebral disc tissues (Kmail 2025; Adoungotchodo 2021; Chen 2025; Zhao 2022).

Blood Vessel and Heart Tissue Engineering

Vascular tissue engineering aims to overcome limitations of synthetic and autologous grafts, such as thrombosis, calcification, and limited remodeling capacity, with recent strategies emphasizing biodegradable and bioactive scaffolds supporting endothelialization and vascular remodeling (Li 2023; Das 2024; Shakeel 2023; Lang 2024). Chitosan, a cationic polysaccharide with a glycosaminoglycan-like structure, has been widely investigated due to its biocompatibility, tunable degradation rate, hemostatic activity, and similarity to native extracellular matrix components, promoting cell adhesion and proliferation (Haider 2024; Kim 2023; Pramanik 2024).

Incorporation of chitosan into hybrid scaffolds has yielded significant advances, with chitosan-gelatin bilayer scaffolds showing improved mechanical stability and cytocompatibility supporting endothelial and smooth muscle cell integration, electrospun chitosan-collagen-PLLA small-diameter vascular grafts demonstrating enhanced tensile strength, hemocompatibility, and cell viability, and porous chitosan scaffolds containing glycosaminoglycans enhancing endothelialization and reducing neointimal hyperplasia (Leal 2021; Fiqrianti 2022; Tan 2023). Chitosan hydrogels and derivatives, including COS, exhibit controlled degradation and bioactive behavior promoting angiogenesis and vascular regeneration, with COS modulating blood compatibility by influencing erythrocyte and platelet activity without inducing hemolysis or coagulation abnormalities, underscoring their potential as multifunctional biomaterials supporting both hemocompatibility and regenerative remodeling (Wang Q 2023; Hsieh 2017; Anil 2022; Guo 2018).

From a cardiovascular perspective, chitosan- based vascular scaffolds address critical clinical challenges in treating cardiovascular diseases (CVD), including atherosclerosis, myocardial infarction, and vascular stenosis (Li 2023; Shakeel 2023; Lang 2024). Chitosan-based vascular scaffolds enhance endothelial cell migration and proliferation, promoting re-endothelialization of damaged vessel walls and reducing thrombotic complications, while their immunomodulatory properties suppress excessive inflammatory responses characteristic of post-intervention restenosis and atherosclerotic progression (Cassano et al. 2024; Zhang W et al. 2024). Furthermore, chitosan's ability to deliver cardioprotective molecules—including anticoagulants, anti-inflammatory agents, and growth factors promoting myocardial angiogenesis—positions it as a promising platform for cardiac tissue regeneration and prevention of ischemic-reperfusion injury (Wang X et al. 2023; Zhang X et al. 2024; Liu et al. 2018).

Chitosan derivatives, particularly chitooligosaccharides (COS), have demonstrated multiple beneficial effects on cardiovascular health. In patients with coronary heart disease (CHD), COS consumption improved left-ventricular ejection fraction, enhanced antioxidant enzyme levels (e.g., superoxide dismutase, glutathione), and helped normalize lipid profiles, likely via increased beneficial gut microbiota such as Lactobacillus and Lactococcus (Jiang et al., 2019). In mouse models of heart failure, COS administration reduced left-ventricular hypertrophy, suppressed inflammation, and improved cardiac function, indicating anti-inflammatory and cardioprotective effects (Zhang et al., 2022). Chitin oligosaccharides attenuated atherosclerosis progression by regulating lipid metabolism and suppressing inflammatory responses in $ApoE^{-/-}$ mice, slowing plaque development (Zhen et al., 2022). Specific COS with degree of polymerization (DP) > 4, such as chitoheptaose, exhibited strong cardioprotective activity in a rat myocarditis model by decreasing inflammatory cytokines, reducing oxidative stress, and preventing cardiac cell death (Xie et al., 2019). In cardiac tissue engineering, chitosan derivatives have been explored as supportive scaffolds for three-dimensional constructs, underscoring their relevance in regenerative cardiovascular applications (Salem et al., 2022). Furthermore, reviews of marine-derived natural products identify chitosan-based compounds as contributors to cardioprotective strategies through modulation of inflammation and vascular function (Liang et al., 2021).

CONCLUSION

Chitosan has emerged as a versatile and promising biomaterial in tissue engineering due to its biodegradability, biocompatibility, structural similarity to glycosaminoglycans, and tunable physicochemical properties, demonstrating capacity to support cell adhesion, proliferation, and extracellular matrix synthesis with favorable mechanical and biological performance across cartilage, bone, intervertebral disc, and vascular engineering applications.(Sections reviewed) Composites with natural polymers, synthetic polymers, ceramics, and nanomaterials have further enhanced scaffold properties enabling constructs that better mimic native tissues. Despite these advances, several challenges remain including limitation of studies to in vitro or small animal models with scarce in vivo and clinical data, rapid degradation, insufficient

long-term mechanical stability, and incomplete host tissue integration, while balancing scaffold strength and biodegradability remains difficult to optimize particularly for load-bearing tissues. Future research should focus on developing multifunctional chitosan-based scaffolds with controlled degradation, improved bioactivity, and tailored mechanical properties, with integration of bioactive molecules, stem cell delivery, and advanced fabrication techniques such as 3D bioprinting and electrospinning potentially providing new opportunities to enhance regenerative outcomes, supporting chitosan's potential to become a key biomaterial in next-generation tissue engineering therapies with further refinement and clinical validation.

Conflict of Interest
Authors must disclose any personal circumstances or interests that could influence the representation or interpretation of the research findings. If no conflicts exist, include the statement: 'None.' Additionally, any influence by funding sponsors on the selection of the research project, study design, data collection, analysis, interpretation, manuscript preparation, or publication decision must be disclosed in this section. Projects funded by industry must ensure a complete declaration of sponsor involvement. If there is no sponsor involvement, state: 'The sponsors had no role in the design, execution, interpretation, or writing of the study.'

Ethical approval
N/A.

Funding disclosure
The funding disclosure must specify any research grants received, including the date, grant number, and the name of the funding institution. If no funding was received, please state “N/A”.

Author contribution
Contributors must provide a detailed delineation of their respective contributions to the manuscript. These contributions should be categorized into the following sections: conception and design, analysis and interpretation of data, drafting of the article, critical revision for important intellectual content, final approval of the article, provision of study materials or patients, statis- tical expertise, securing funding, administrative, technical, or logistical support, and data collection and assembly. The authors' contributions will be published alongside the paper. At least one author must assume responsibility for the integrity of the entire work from inception to publication and should be designated as a 'guarantor.' Further details can be found in the Authors’ Declaration & CTA form. Please detail each author's contribution accordingly. For example, VL contributed to the conception and design, drafting of the article, obtainment of funding, and final approval of the article. MF carried out the collection, assembly, analysis, and interpretation of the data, as well as provided administrative and technical support.

Data availability
N/A.

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