REVIEW

NANOSIZED CHITOSAN SYSTEMS FOR REGENERATIVE MEDICINE

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Abstract

Nanoscience and nanotechnology have revolutionised medicine, leading to groundbreaking advancements in drug delivery, gene therapy, and tissue regeneration. Among nanomaterials, nanoparticles stand out due to their high surface-to-volume ratio and tunable physicochemical properties. Chitosan, a biopolymer derived from chitin, has attracted considerable attention for its biocompatibility, biodegradability, and antimicrobial and antioxidant properties. However, its limited solubility necessitates chemical modifications to enhance its biomedical applicability. Recently, nanosized chitosan has emerged as a promising material for regenerative medicine, offering improved mechanical and biological properties that support tissue repair. This review examines the use of chitosan nanoparticles in regenerative medicine, with a focus on their applications in bone and cartilage regeneration, cardiac tissue repair, skin regeneration and wound healing, nervous system repair, and drug and gene delivery. By highlighting recent advancements and existing challenges, this review analyses the potential of chitosan-based nanosystems as a versatile and effective biomaterial for regenerative therapies.

Keywords: *nanotechnology, chitosan nanosystems, nanomedicine, regeneration, wound healing, drug delivery*

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1. Introduction

Nanoscience and nanotechnology have become increasingly significant in recent decades, drawing the attention of researchers worldwide. The application of nanometre-scale materials and devices is transforming various scientific and industrial fields, offering innovative solutions to technological and environmental challenges [1]. According to the Royal Society and the Royal Academy of Engineering Working Group, nanoscience focuses on the study of structures at the atomic and molecular scales, while nanotechnology applies this knowledge to develop and manipulate devices and systems controllable at the nanometric level [2]. The origins of nanoscience can be traced back to ancient Greece, where philosophers first hypothesised the existence of indivisible particles known as atoms [3]. A pivotal contribution came from physicist Richard Feynman, who introduced the concept of nanotechnology in his renowned 1959 lecture "There's Plenty of Room at the Bottom," suggesting the possibility of manipulating individual atoms and molecules to construct novel structures [4]. However, the practical advancement of nanotechnology began in the 1980s with the invention of the scanning tunnelling microscope (STM) and the atomic force microscope (AFM), which allowed scientists to directly observe and manipulate atoms, marking a transformative milestone in the field [5, 6]. In the early 2000s, progress accelerated significantly, particularly after the enactment of the "21st Century Nanotechnology Research and Development Act" in 2003 by U.S. President George W. Bush, which formally recognised nanotechnology as a national research priority [7]. One of the most significant advancements in this field has been the development of nanoparticles - particles smaller than 100 nm, either amorphous or crystalline in structure, composed of millions of atoms or molecules of the same or different types [8]. These particles occupy an intermediate state between macroscopic materials and atomic or molecular structures, representing a distinct phase of matter with unique physical, chemical, and biological properties compared to their bulk counterparts [9]. Among their most remarkable features is their high surface-to-volume ratio, which enhances chemical reactivity and facilitates molecular interactions [9]. Their ease of functionalisation further enhances their applicability, particularly in nanomedicine, where they play a crucial role in targeted drug delivery to specific tissues [10]. In this context, polymeric nanoparticles composed of natural polymers, with sizes typically ranging from 10 nm to 1 μm, are particularly advantageous for controlled drug release and the optimisation of targeted delivery systems thanks to their high biocompatibility, stability, water solubility, and extended shelf life [11]. These biopolymers offer numerous advantages, such as being sourced from renewable marine or agricultural food materials, their biological tolerance and biodegradability, which contribute to environmental safety, and their adaptability for producing diverse chemically or enzymatically modified derivatives for different applications [12]. Recently, natural polymer compounds have been extensively utilised across fields such as biology, medicine, cosmetics, and healthcare [13]. Among these, chitosan has emerged as a topic of particular interest [14]. Chitosan is a natural polymer obtained through the deacetylation of chitin, a substance found in the exoskeletons of crustaceans such as shrimp and crabs. On a molecular level, its complex structure consists of distinctively arranged β-(1,4)-linked-D-glucosamine and *N*-acetyl-glucosamine units, making them the only naturally occurring forms of these glucosamine units. This remarkable composition grants it a variety of valuable biological properties, including antimicrobial effects, antioxidant activity, adhesive abilities, and exceptional biodegradability [15]. Chitosan is a safe, well-adsorbing, and renewable substance that does not have any adverse effects. According to the US Food and Drug Administration (FDA), it is safe for use in food and medication [16]. The solubility of

chitosan in various solvents is notably limited, as it can only dissolve in a few dilute acid solutions, which hinders its applications. Consequently, efforts to chemically modify chitosan to improve its solubility and broaden its potential applications have become a focus of attention [17]. Chitosan possesses three reactive sites: hydroxyl groups located at carbon positions 3 and 6, as well as an amino group attached to the carbon at position number 2 in each deacetylated unit. These functional groups are highly reactive and can undergo chemical modifications, leading to alterations in the mechanical and physical properties of chitosan [18, 19]. Chitosan can undergo etherification or esterification reactions on its hydroxyl groups (*O*-substitution) by protecting the amino group through the formation of a Schiff base or an *N*-phthaloyl functional group. Additionally, when alkyl halides or acid chlorides are used in reactions with chitosan, the nucleophilic nature of the amino group allows for selective *N*-substitution, such as *N*-alkylation and *N*-acylation [20, 21]. Furthermore, numerous studies have focused on the chemical modification of chitosan through its amine group, as these modifications significantly improve its solubility and enhance its suitability for various applications [22, 23]. Similarly, chemical modification not only improves the chemical and physical properties of chitosan but also enhances its unique characteristics and expands its range of applications [24]. Additionally, modified chitosan derivatives retain key pharmacological properties - including antibacterial, antiviral, and biodegradable activities - while exhibiting enhanced biocompatibility and bioactivity, without posing safety risks [25, 26]. Given these, chitosan can be regarded as a promising compound for innovative therapeutic approaches such as regeneration.

2. Regenerative Medicine

The loss of organs and tissues due to injury or disease has driven the development of innovative therapies aimed at tissue regeneration, reducing dependence on transplants. Regenerative medicine focuses on restoring damaged tissues and organs by integrating fundamental cell biology with advanced engineering techniques. This field presents a promising alternative to transplantation, addressing challenges such as limited donor availability and immune system complications. Applications include bone regeneration, cardiac therapy, and muscle tissue repair. Given that cellular activities take place on a nanometre scale, nanotechnology offers the potential to modulate and enhance cellular behaviour, ultimately improving tissue and organ function. Various nanotechnology-based approaches in regenerative medicine include the use of nanoparticles, nanofibre scaffolds, nano-topographic modifications in scaffolds, drug and gene delivery systems, and extracellular matrix (ECM) patterning. Recent advancements often combine multiple traditional strategies to achieve better therapeutic outcomes [27]. Among biomaterials, chitosan has gained significant attention in regenerative medicine due to its capacity to promote tissue repair and regeneration. A growing body of research highlights the application of chitosan formulations in regenerating various tissues, including bone, cartilage, dental, corneal, skin, nervous, and cardiac tissues. For instance, chitosan-based biomaterials have been found to enhance mineralisation, stimulate osteogenesis, and facilitate bone defect repair in animal models of bone injury [28]. Additionally, chitosan formulations support chondrocyte proliferation and differentiation, promote chondrogenesis, enhance the production of cartilage-specific proteins, and contribute to the development of new cartilage tissue [29]. Chitosan-based materials have also demonstrated beneficial effects in cardiovascular regeneration. Hydrogels containing chitosan have been shown to prevent adverse cardiac remodelling and improve heart

function in experimental models of cardiomyopathy and myocardial infarction. Furthermore, specific chitosan composites have been effective in facilitating electrical conduction, which plays a crucial role in myocardial tissue regeneration [30]. In skin regeneration and wound healing, chitosan is particularly valuable due to its antimicrobial and haemostatic properties [11, 31]. Research has shown that chitosan-based biomaterials can promote cell proliferation at wound sites, support complete wound regeneration, and aid in epithelial tissue reconstruction [32].

3. Nanosized Chitosan: Synthesis and Properties

3.1. Synthesis of Chitosan Nanosystems

Nanotechnology has facilitated the development of chitosan-based nanostructures with improved physicochemical and biological properties, making them highly valuable in various biomedical, pharmaceutical, and industrial applications. Nanosized chitosan can be synthesised through different methodologies, which are generally classified into top-down and bottom-up approaches. The top-down approach primarily involves the mechanical or chemical degradation of bulk chitosan to achieve nanoscale particles [33], while the bottom-up methods focus on the self-assembly of molecular units into nanostructures through controlled interactions and specific reaction conditions [34]. The choice of synthesis method plays a crucial role in determining the final properties of chitosan nanoparticles, including their size, morphology, stability, and bioactivity, which in turn influence their functionality in targeted applications such as wound healing, drug delivery, and antimicrobial treatments. Among the most commonly employed methods, ionic gelation has gained significant attention due to its simplicity, mild reaction conditions, and ability to produce stable nanoparticles without the need for harsh chemicals or extreme temperatures [35]. This technique relies on the electrostatic interaction between the positively charged chitosan and negatively charged polyanions, such as tripolyphosphate (TPP), resulting in the formation of nanosized particles with well-defined structures and high biocompatibility [36]. Another widely used technique is nanoprecipitation, which involves the rapid blending of the chitosan solution with a non-solvent, leading to the displacement of the solvent and subsequent formation of nanoparticles through spontaneous self-assembly. This method is particularly advantageous for obtaining nanoparticles with uniform size distribution and high reproducibility [37]. In addition to these, emulsion-based techniques, including water-in-oil (W/O) and oil-in-water (O/W) emulsions, have been extensively utilised to synthesise chitosan nanoparticles, especially for applications in drug delivery and controlled release systems [38]. These approaches enable the formation of well-structured nanoparticles with precise control over surface characteristics, dimensions, and encapsulation efficiency, allowing for enhanced drug loading and sustained release profiles. Furthermore, mechanical techniques such as ultrasonication and high-pressure homogenisation provide effective means to reduce chitosan particle size and achieve nanoscale dimensions by applying intense shear forces and cavitation effects. These physical methods are particularly useful for producing nanoparticles with improved solubility and bioavailability while maintaining the inherent biocompatibility and biodegradability of chitosan [39, 40]. Figure 1 is a schematic synthesis of chitosan nanoparticles (CNPs) by ionotropic gelation.

In addition, nanosized chitosan electrospun fibres have garnered significant attention in recent years for a wide range of biomedical and environmental applications. The synthesis of these nanofibers typically involves the electrospinning technique, wherein

chitosan is blended with co-polymers such as poly(ethylene oxide) (PEO) or poly(vinyl alcohol) (PVA) to enhance its spinnability, owing to its intrinsic poor solubility and high viscosity in aqueous solutions. Critical parameters influencing fibre morphology and diameter include solution concentration, applied voltage, flow rate, and ambient conditions. Advances in electrospinning setups, such as coaxial and multi-jet systems, have further enabled the production of uniform, defect-free fibres with controlled porosity and functional surface properties. Beyond nanoparticles and electrospun fibres, nanosized chitosan can be fabricated into various advanced structures such as nanofilms, nanocoatings, nanosponges, and nanotubes, each with distinct synthesis techniques and applications. Nanofilms, often produced via layer-by-layer assembly or spin coating, yield ultrathin, biocompatible layers ideal for biosensing and wound dressings. Nanocoatings, achieved through dip-coating or plasma deposition, provide antimicrobial and protective surfaces on implants and packaging materials. Nanosponges, synthesised by crosslinking chitosan into porous 3D networks, are valued for their high loading capacity in drug delivery or pollutant adsorption. Chitosan nanotubes, generated using templating methods, offer high aspect ratios beneficial for tissue engineering and controlled release systems [39, 40].

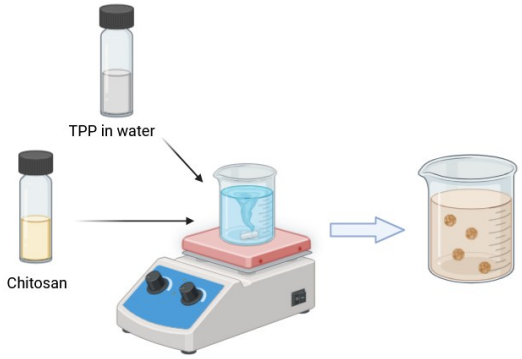

Figure 1. CNPs synthesis by ionotropic gelation. Image realised with BioRender.

3.2. Key Properties of Nanosized Chitosan

The nanoscale dimension of chitosan significantly enhances its physicochemical, structural, and biological properties, making it an exceptional material for biomedical applications, especially in regenerative medicine, wound healing, drug delivery, and tissue engineering. An important advantage of nanosized chitosan is enhanced biocompatibility and bioactivity, which stem from its natural origin and structural similarity to extracellular matrix components. At the nanoscale, chitosan exhibits an increased surface-to-volume ratio, allowing for greater interaction with biological systems, which in turn promotes cellular adhesion, proliferation, and differentiation [41]. This characteristic is particularly beneficial in tissue engineering applications, where chitosan-based nanostructures can serve as bioactive scaffolds supporting cell growth and tissue regeneration [42]. In addition to nanoparticles, other nanosized chitosan formats such as nanofibers, nanohydrogels, and nanospheres have gained significant attention due to their versatility. For instance, electrospun chitosan nanofibers provide highly porous, ECM-mimicking architectures that support cellular alignment and migration, making them ideal for neural, skin, and vascular regeneration. Similarly, nanohydrogels formed through physical or chemical crosslinking offer soft, hydrated environments suitable for encapsulating and releasing growth factors, cytokines, or cells in a controlled fashion. These systems also

allow for injectable formulations, increasing their clinical usability. Additionally, the nanosized form improves the material's ability to interact with proteins, enzymes, and cellular receptors, facilitating biological responses that contribute to wound healing and antimicrobial activity [43]. Another critical feature of nanosized chitosan is its high surface area and functionalisation potential, which arises from the abundance of amine ($–NH_2$) and hydroxyl (–OH) functional groups along its polymer backbone. These substituents not only contribute to chitosan's inherent bioactivity but also enable extensive chemical modifications, allowing the tuning of its properties for specific applications. By modifying the polymer chains through processes such as acetylation, grafting, or conjugation with bioactive molecules, scientists can enhance chitosan's solubility, bioavailability, and ability to bind to target molecules, making it a versatile material for drug delivery and biomedical engineering [44]. Furthermore, the high surface area allows for the adsorption and immobilisation of therapeutic agents, bioactive molecules, or nanoparticles, expanding its applicability in nanomedicine, diagnostics, and biosensing technologies [45]. From a structural and mechanical perspective, nanosized chitosan exhibits superior mechanical strength and stability. Compared to bulk chitosan, its nanoscale counterpart demonstrates improved flexibility, porosity, and elasticity, which are crucial for supporting tissue regeneration, cell infiltration, and vascularisation [46]. Moreover, the reduced particle size enhances the polymer's ability to disperse uniformly within composite materials, resulting in more homogeneous structures that can better mimic the natural extracellular matrix. These mechanical advantages are not restricted to nanoparticles. Nanofiber mats and nanohydrogels can also be engineered to exhibit tunable stiffness and degradation profiles, important for dynamic tissue environments such as bone and cartilage repair. Incorporation of nanosized chitosan within composite scaffolds further improves load-bearing capacity while maintaining bioactivity. These properties make nanosized chitosan highly desirable for bone regeneration, cartilage repair, and skin tissue engineering, where mechanical integrity is essential for long-term functionality [47]. Perhaps one of the most widely explored applications of nanosized chitosan lies in its role as an advanced drug delivery system. Chitosan nanoparticles serve as highly efficient carriers for sustained, controlled, and targeted drug delivery, leading to optimised therapeutic effects and decreased systemic side effects. The polymer's natural mucoadhesive properties enable it to adhere to biological membranes, facilitating prolonged retention and improved drug absorption across mucosal barriers such as the gastrointestinal tract, nasal passages, and ocular surfaces [48]. Furthermore, the ability of chitosan nanoparticles to respond to environmental stimuli, such as pH changes or enzymatic activity, makes them ideal for site-specific drug release in conditions like cancer therapy, infection treatment, and gene delivery [49]. The presence of functional groups on chitosan molecules also allows for the encapsulation of hydrophobic and hydrophilic drugs, expanding its use for delivering a wide range of pharmaceuticals, including antibiotics, anticancer agents, proteins, and nucleic acids [50]. Similarly, chitosan nanohydrogels have demonstrated promise as platforms for dual or sequential drug release, and chitosan nanofibers have been developed as local delivery systems for antibiotics or anti-inflammatory agents in chronic wound dressings. These nanosystems offer localised, minimally invasive delivery strategies that complement the systemic delivery advantages of nanoparticles. Figure 2 shows some of the chitosan derivatives used for biomedical applications.

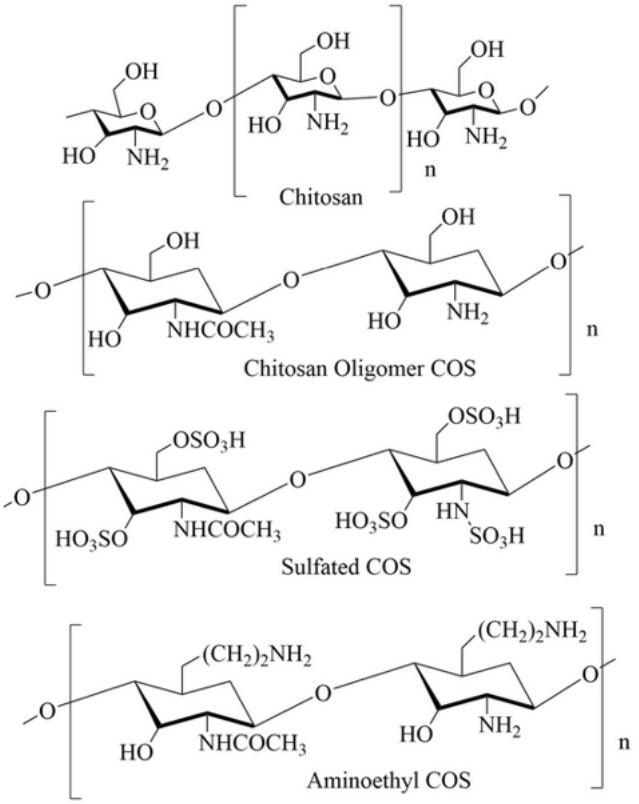

Figure 2. Chitosan derivatives. From top to bottom, chitosan, chitosan oligomer (COS), sulfated COS and aminoethyl COS.

4. Applications of Nanosized Chitosan in Regenerative Medicine

4.1. Bone and Cartilage Regeneration

Chitosan nanoparticles have been extensively studied for their applications in bone and cartilage regeneration. Their osteoconductive and osteoinductive properties contribute to mineralisation, osteogenesis, and the healing of bone defects. Research findings indicate that chitosan-based scaffolds effectively support chondrocyte differentiation, stimulate chondrogenesis, and enhance the secretion of cartilage-specific proteins, leading to the development of new cartilage tissue. Additionally, loading chitosan nanoparticles with growth factors or osteoinductive agents further improves their regenerative capabilities. Numerous studies exemplify these applications. Alshubaily and Jambi investigated the role of shilajit water extract (SWE) in bone development and osteoporosis management using a glucocorticoid-induced osteoporotic rat model. To optimise the therapeutic effects of SWE, they synthesised nanochitosan (NCT) and developed an SWE/NCT conjugate. Their study assessed the efficacy of these formulations in treating osteoporosis, reporting that NCT and SWE/NCT exhibited mean particle sizes of 196.4 nm and 248.4 nm, respectively, with strong positive surface charges and stability. The biochemical and anti-osteoporotic effects of SWE and its conjugated form were examined in different groups of osteoporotic rats [51]. In another study, Takanche et al. engineered a plasmid DNA/c-myb system conjugated with chitosan-gold nanoparticles (Ch-GNPs/c-myb) to

enhance osteogenesis while inhibiting osteoclastogenesis in MC-3T3 E1 cells. Their findings revealed that Ch-GNPs/c-myb downregulated key osteoclastogenic markers, including nuclear factor of activated T-cells 1 (NFATc1), c-Fos, and tartrate-resistant acid phosphatase (TRAP)-positive multinucleated osteoclasts in receptor activator of nuclear factor-κB ligand (RANKL)-stimulated bone marrow macrophages. Furthermore, in vivo experiments on rat mandibles demonstrated that titanium (Ti) implants coated with Ch-GNP/c-myb significantly improved bone volume, density, and osseointegration in dental implants, as assessed via micro-computed tomography in an osteoporosis model induced by ovariectomy (OVX). Immunohistochemical analysis confirmed increased c-myb expression and upregulation of bone morphogenetic proteins (BMPs), osteoprotegerin (OPG), and EphB4, alongside reduced RANKL expression. Haematoxylin and eosin staining further validated the enhanced new bone formation surrounding the implants [52]. Abouelseoud *et al*. explored the effects of Lepidium Sativum L. extract (LS) encapsulated within chitosan nanoparticles on the gene expression of miR-142-3p and miR-23a in a rat model of osteoporosis. Osteoporosis was induced via subcutaneous administration of methylprednisolone (3.5 mg/kg body weight/day) over four weeks. The osteoporotic rats were then treated orally for 12 weeks with LS extract (400 mg/kg body weight/day), chitosan nanoparticles (CN), or LS extract-loaded CN (400 mg/kg body weight/day). Their findings showed that treatment with LS, CN, and LS-loaded CN led to varying degrees of improvement in biochemical markers associated with bone health, including enhanced serum calcium and phosphorus levels. The treated rats exhibited a significant reduction in miR-23a expression and an upregulation of miR-142-3p expression, suggesting an improvement in osteoporosis. Histopathological analysis further supported the protective effects of LS and LS-loaded CN on bone integrity, highlighting their potential as safe and effective therapeutic agents for osteoporosis management [53]. In another study, Chen *et al*. developed a chitosan-based bone-targeted delivery system designed to enhance the therapeutic effects of Cyclolinopeptide J (CLJ), a bioactive peptide derived from flaxseed, for osteoporosis treatment. They engineered bone-targeting polymer conjugates (CSD8) by crosslinking carboxylated chitosan (CMCS) with the functional peptide (ASP8). These conjugates were then modified onto the surface of CLJ-loaded nanoparticles, forming the JCA/CSD8 system. The JCA/CSD8 nanoparticles were reported to have a particle size of 122.4 ± 1.8 nm and a CLJ loading capacity of 22.7%. The study demonstrated that JCA/CSD8 exhibited 11.7-fold higher *in vitro* bone affinity and 13.6-fold greater *in vivo* bone-targeting efficiency compared to unmodified nanoparticles. Additional *in vitro* findings showed that JCA/CSD8 degraded in lysosomes under acidic conditions, releasing CLJ and Ca^{2+}, thereby promoting osteogenesis through targeted delivery. Gene expression analysis indicated that JCA/CSD8 upregulated key osteogenic markers such as OPG, Col-I, OCN, OPN, RUNX2, and ALP, confirming its potential for bone regeneration. In an OVX-induced osteoporosis mouse model, JCA/CSD8 treatment significantly increased bone density and restored trabecular bone structure, surpassing the results obtained in the positive control group [54]. Zohri *et al*. assessed the regenerative potential of chitosan/alginate nanoparticles loaded with recombinant human bone morphogenetic protein-2 (rhBMP-2) and an SMAD4-encoding plasmid to promote chondrogenesis in human bone marrow mesenchymal stem cells (hBM-MSCs) seeded on an extracellular matrix (ECM). Their study included multiple experimental groups: a biological cocktail (BC) group, a negative control (NC) group, hBM-MSCs with a chondrogenic medium (MCM), hBM-MSCs treated with rhBMP-2 and chondrogenic medium (NB/C), and hBM-MSCs treated with rhBMP-2, chondrogenic medium, and an SMAD4-encoding

plasmid transfected with polyethyleneimine (PEI) (NB/C/S/P). Cartilage differentiation was evaluated using real-time quantitative PCR analysis and alizarin blue staining. The results demonstrated that the BC group significantly upregulated cartilage-specific gene expression compared to the MCM and NC groups, highlighting its superior chondrogenic potential. In the NB/C/S/P group, SOX9 and COLX expression levels were lower than those observed in the BC group. The expression pattern of the ACAN gene closely mirrored that of COL2A1, suggesting a role in cartilage regeneration [55]. Table 1 provides a summary of these studies.

Table 1. Chitosan nanosystems in bone and cartilage regeneration.

Device	Characteristics	Reference
Nanochitosan particles with shilajit water extract	Regenerative potential in managing osteoporosis in experimental glucocorticoid-induced osteoporotic rats	[51]
Chitosan-gold nanoparticles conjugated with plasmid DNA/c-myb	Reduction of the expression NFATc1, c-Fos, and TRAP-positive multinucleated osteoclasts	[52]
Lepidium sativum L. Extract (LS) loaded onto chitosan nanoparticles	Osteoporosis management	[53]
Chitosan nanoparticles with Cyclolinopeptide J	Increased bone density and restoration of trabecular bone architecture	[54]
Chitosan-alginate nanoparticles containing plasmids (recombinant human bone morphogenetic-2 and a SMAD4-encoding)	Superior chondrogenic potential with potential for cartilage regeneration	[55]

4.2. Cardiac Tissue Regeneration

Chitosan-based nanomaterials have emerged as key components in cardiac and vascular tissue regeneration. Hydrogels embedded with chitosan nanoparticles have demonstrated potential in mitigating detrimental cardiac remodelling and enhancing myocardial function in models of cardiomyopathy and myocardial infarction. Additionally, some composite chitosan formulations facilitate electrical conductivity, a critical factor in heart tissue repair. For instance, Wang *et al*. investigated the impact of co-administering basic fibroblast growth factor (bFGF) with a thermosensitive chitosan nanoparticle hydrogel in a rat model of myocardial infarction. Their findings indicated a marked improvement in cardiac function, evidenced by enhanced left ventricular performance and greater arteriole density in infarcted regions compared to controls. Moreover, the chitosan+bFGF group exhibited significantly reduced infarct size and fibrosis [56]. Similarly, Wang *et al*. designed a cardiac patch incorporating calcium silicate (CS) into chitosan-based electrospun nanofibers, aiming to advance cardiac tissue engineering. The presence of silicon ions within an optimal concentration range significantly upregulated cardiac-specific gene expression and promoted neonatal rat cardiomyocyte (NRCM) proliferation. This bioengineered scaffold provided both biochemical and structural stimuli, fostering aligned cellular morphology, enhanced cell viability, organised myofilament structures, and improved calcium transients relative to non-CS or randomly aligned scaffolds. *In vivo* studies revealed that NRCM-seeded aligned CS/chitosan patches contributed to improved

cardiac function by reducing scar tissue and promoting angiogenesis following myocardial infarction [57]. Shu *et al*. introduced a thermosensitive chitosan chloride-RoY (arg-tyr 12 aminoacids peptide- CSCl-RoY) nanohydrogel to stimulate angiogenesis under hypoxic conditions post-myocardial infarction. RoY peptides were conjugated to the CSCl polymer chain via amide bonds, preserving the hydrogel's temperature-responsive properties. *In vitro* experiments using human umbilical vein endothelial cells (HUVECs) demonstrated that CSCl-RoY hydrogels significantly enhanced cell survival, proliferation, migration, and tube formation under hypoxic conditions compared to CSCl hydrogels alone. Further analysis indicated that CSCl-RoY modulated GRP78 receptor expression while activating the Akt and ERK1/2 signalling pathways, thereby augmenting angiogenesis. *In vivo* injections of the hydrogel into infarcted rat hearts resulted in enhanced vascularisation and improved myocardial function [58]. Wardani *et al*. explored the cardioprotective properties of chitosan nanoparticles in streptozotocin-induced diabetic rats by assessing their effects on oxidative stress and apoptosis. Characterisation via scanning electron microscopy (SEM) and dynamic light scattering (DLS) showed that the nanoparticles exhibited an irregular shape, rough surface, and an average diameter of 247.3 ± 38.1 nm. Their experimental design included control, streptozotocin-induced, and chitosan nanoparticle-treated groups receiving varying doses. Streptozotocin administration led to increased levels of CK-MB, LDH, MDA, and Caspase-3 while decreasing SOD, GPx, Nrf2, and Bcl-2, contributing to cardiac structural damage and necrosis. Conversely, chitosan nanoparticle treatment significantly reversed these effects, reducing CK-MB, LDH, MDA, and Caspase-3 expression while increasing SOD, GPx, Nrf2, and Bcl-2 levels. These results suggest that chitosan nanoparticles provide cardioprotection in diabetic conditions by mitigating oxidative stress through ROS reduction and upregulation of Nrf2, while also inhibiting apoptosis via increased Bcl-2 and reduced Caspase-3 expression [59]. Hosny *et al*. evaluated the therapeutic efficacy of alpha-lipoic acid (α-LA) and caffeine-loaded chitosan nanoparticles (Caf-CNs) in counteracting cardiovascular complications associated with obesity. Their study involved control, high-fat diet (HFD)-induced obesity, and treatment groups administered α-LA and/or Caf-CNs. Obese rats exhibited elevated levels of triglycerides (TG), total cholesterol (TC), LDL-C, VLDL-C, IL-1β, TNF-α, LDH, CK-MB, and several atherogenic indices, alongside increased malondialdehyde (MDA), nitric oxide (NO), and monoamine oxidase (MAO) activity in cardiac tissue. Concurrently, Na+/K+-ATPase and acetylcholinesterase (AchE) activities, as well as GSH, serotonin (5-HT), norepinephrine (NE), dopamine (DA), and HDL-C levels, were significantly decreased. Treatment with α-LA and/or Caf-CNs effectively restored most of these biochemical and histopathological parameters, suggesting their potential role in cardiovascular protection [60]. The summarised results are presented in Table 2.

4.3. Skin Regeneration and Wound Healing

Due to its haemostatic and antimicrobial properties, nanosized chitosan has demonstrated significant efficacy in wound healing and skin regeneration. Research has shown that chitosan nanoparticles promote cell proliferation, expedite wound closure, and support epithelial reconstruction, making them ideal candidates for advanced wound dressings and skin graft applications. Additionally, chitosan nanoparticles carrying bioactive agents, such as antimicrobial peptides and growth factors, can further enhance the wound healing process. For instance, Zhang and Liu developed a novel recombinant human epidermal growth factor (rhEGF) delivery system utilising self-assembled amphiphilic polymers to

Table 2. Chitosan nanosystems in cardiac tissue regeneration.

Device	Characteristics	Reference
Temperature-responsive chitosan nanosized hydrogel loaded with fibroblast growth factor (bFGF)	Increased arteriole density in the infarcted area with enhanced left ventricular function	[56]
Calcium silicate into aligned chitosan electrospun nanofibers	Stimulation of cardiac-specific gene expression	[57]
Nanohydrogel of Chitosan chloride loaded with RoY (arg-tyr 12 aminoacids peptide)	Ability to enhance angiogenesis under hypoxia provoked by myocardial infarction	[58]
Chitosan nanoparticles	Protection against diabetic cardiac damage	[59]
Chitosan nanoparticles loaded with alpha-lipoic acid and caffeine (Caf-CNs)	Action against cardiovascular damage induced by obesity	[60]

improve its bioavailability and controlled release. To protect rhEGF from enzymatic degradation, they engineered nanoparticles consisting of conjugated linoleic acid (CLA) and carboxymethyl chitosan (CMCS), characterised through Fourier-transformed infrared spectroscopy (FTIR) and ^{1}H nuclear magnetic resonance (^{1}H NMR). Their study revealed that the self-assembly of CLA–CMCS (CC) in water resulted in a reduction in particle size from 196 to 155 nm as CLA substitution increased. The nanoparticles achieved a maximum rhEGF loading efficiency of 82.43 ± 3.14% without cytotoxic effects on L929 cells, preserving rhEGF's ability to stimulate proliferation. Topical application of rhEGF:CC-NPs significantly promoted wound healing in full-thickness skin wounds, attributed to their sustained release and enhanced skin penetration [61]. Similarly, You *et al*. designed a hybrid scaffold incorporating metallic nanosilver particles into a collagen/chitosan matrix (NAg-CCS) to address challenges associated with full-thickness skin defect repair, such as infection risks and excessive scarring. Silver nanoparticles (NAg) are well known for their antimicrobial properties, yet their precise mechanism in wound healing requires further investigation. Their research included *in vitro* scratch assays, immunofluorescence staining, and antibacterial assessments, alongside *in vivo* studies in Sprague-Dawley (SD) rats with full-thickness skin defects. Results demonstrated that NAg at 10 ppm enhanced fibroblast migration and increased α-smooth muscle actin (α-SMA) expression. Furthermore, *in vivo* findings indicated that NAg-CCS elevated levels of pro-inflammatory and scar-related factors while upregulating macrophage activation markers. After 60 days, the regenerated skin treated with NAg-CCS closely resembled normal skin structure [62]. Montazeri *et al*. developed epidermal growth factor (EGF)-loaded chitosan nanoparticles to enhance stability under physiological pH and protect EGF's biological activity for wound healing applications. Since EGF is highly susceptible to enzymatic degradation, ensuring a sustained concentration at the wound site presents a challenge. Using a straightforward formulation approach, they produced nanoparticles with various chitosan/EGF ratios and assessed their performance *in vitro* and *in vivo*. Their findings showed that nanoparticles with a 2:1 chitosan/EGF ratio achieved an 80% protein release within 12 hours. Cell proliferation assays confirmed that these nanoparticles maintained EGF functionality under physiological conditions. *In vivo* results further revealed that the nanoparticles significantly accelerated wound closure, re-epithelialisation, and collagen deposition [63].

Qi *et al*. formulated hypaphorine (HYP)-loaded chitosan (CS) nano-microspheres (HYP-NPS) to evaluate their therapeutic potential in treating chronic wounds in diabetic rats. Since impaired wound healing remains a major complication in diabetes, their study aimed to explore the mechanism underlying the effects of HYP-NPS. Transmission electron microscopy (TEM) analysis showed that the nanoparticles were spherical, with an average size of approximately 50 nm. Biocompatibility assessments using RAW 264.7 macrophages suggested that HYP-NPS exhibited significant potential as a wound-healing material. In a diabetic rat model with full-thickness dermal wounds, HYP-NPS accelerated healing by reducing pro-inflammatory cytokines IL-1β and TNF-α, thereby facilitating the transition from inflammation to tissue regeneration [64]. Chitosan nanoparticles have also been investigated for periodontal wound healing. Conte *et al*. developed a novel strategy for treating periodontitis by encapsulating Opuntia ficus-indica extract in chitosan nanoparticles (OFI-NPs) using ionotropic gelation through a microfluidic system, ensuring precise nanoparticle control and protection against enzymatic degradation. Recognising the limitations of conventional treatments in eradicating pathogenic bacteria from deep periodontal pockets, they formulated a thermo-responsive hydrogel comprising hyaluronic acid and Pluronic F127 (OFI@tgels) for localised and sustained drug delivery. This hydrogel remains in a liquid state at lower temperatures and solidifies at body temperature, enabling prolonged release at the site of inflammation. Their optimised formulation exhibited potent antibacterial activity, eradicating biofilms of *S. mutans*, *P. aeruginosa* (PAO1), and *P. gingivalis* over a 36-hour period while disrupting extracellular polymeric substance formation. Furthermore, OFI@tgel modulated immune responses by inhibiting M1 macrophage polarisation and promoting a shift toward the M2 phenotype, facilitating tissue regeneration [65]. Table 3 summarises these applications.

Table 3. Chitosan nanosystems in skin regeneration and wound healing.

Device	Characteristics	Reference
Nanoparticles of carboxymethyl chitosan with conjugated linoleic acid	Acceleration of wound closure	[61]
Metallic nanosilver particle-collagen/chitosan hybrid scaffold	Antibacterial action on full-thickness skin defect treatment	[62]
Chitosan nanoparticles containing epidermal growth factor	Acceleration of epithelial renewal and collagen synthesis	[63]
Chitosan nano-microspheres conjugated with hypaphorine	Promotion of healing by downregulation of pro-inflammatory cytokines	[64]
Opuntia ficus-indica extract into chitosan nanoparticles	Potent antibacterial activity; Disruption of extracellular polymeric substance formation	[65]

4.4. Nervous System Repair

Chitosan nanoparticles have been explored for their potential in nerve regeneration due to their ability to facilitate neural cell attachment and differentiation. Chitosan-based scaffolds have demonstrated effectiveness in guiding neuronal growth, offering neuroprotection, and aiding in the restoration of nerve function following injuries. Additionally, chitosan nanoparticles have been investigated as carriers for neurotrophic factors to enhance nerve repair. For instance, Chen *et al*. reported that chitosan

nanoparticles (~100–200 nm in diameter; 1 mg/ml) successfully reinstated nerve signal transmission in an *in vivo* guinea pig spinal cord injury model. Their study compared subcutaneously injected chitosan nanoparticles with control injections of silica particles of the same size and concentration in a standardised spinal cord injury setup. Physiological assessments of evoked potentials at the sensorimotor cortex following tibial nerve stimulation showed restored conduction exclusively in the chitosan-treated group. Additional investigations into the effects of chitosan's acetylation degree and molecular weight on membrane-sealing properties revealed no significant enhancements in effectiveness. *Ex vivo* dye-exchange membrane assays on injured spinal cord samples similarly showed no notable differences between comparators. Notably, no recovery in nerve conduction was observed in the control group [66]. Wang *et al*. developed valproic acid-conjugated chitosan nanoparticles (VA-CN) as a targeted intervention for spinal cord injury (SCI) and assessed their therapeutic potential. Their findings indicated that VA-CN treatment significantly improved functional recovery and tissue regeneration post-SCI. Furthermore, administration of VA-CN reduced reactive astrocyte activation and increased neuronal marker NF160 expression, suggesting a neuroprotective role in SCI models. Pro-inflammatory cytokine levels, including IL-1β, IL-6, and TNF-α, were markedly reduced following VA-CN treatment, highlighting its anti-inflammatory effects. Additionally, VA-CN administration mitigated blood spinal cord barrier (BSCB) disruption, further supporting its potential in promoting SCI recovery [67]. Safari *et al*. synthesised chitosan nanoparticles encapsulating naringin to alleviate complications associated with chronic constriction injury (CCI)-induced neuropathic pain. The produced nanoparticles exhibited an average particle size of 220 nm, PDI = 0.37, a near-spherical morphology, and a zeta potential of +41.5 mV. Characterisation confirmed a high encapsulation efficiency and prolonged naringin release. Anti-neuropathic evaluations in CCI-induced rats demonstrated that treatment with naringin-loaded nanoparticles (10 mg/kg) significantly reduced hyperalgesia and cold allodynia. Additionally, the treatment enhanced sensory and motor functions, as evidenced by decreased paw licking, increased rearing behaviour, and improved crossing performance. The nanoparticles effectively reduced elevated nitrite levels and restored glutathione levels in CCI-induced rats. Histopathological analysis further confirmed sciatic nerve repair by minimising myelin degradation, axonal swelling, and nerve fibre disorganisation [68]. Eldeeb *et al*. investigated the neurotoxicity of hydroxyapatite nanoparticles (HANPs) and the potential neuroprotective effects of chitosan nanoparticles (CNPs) and curcumin nanoparticles (CUNPs). Their study found that HANPs significantly decreased neurotransmitter levels, including acetylcholine (Ach), dopamine (DA), serotonin (SER), epinephrine (EPI), and norepinephrine (NOR). Moreover, HANPs strongly suppressed the cortical expression of mitochondrial biogenesis-related genes such as peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) and mitochondrial transcription factor A (mTFA). The findings also highlighted severe neuroinflammation, increased apoptosis, lipid peroxidation, oxidative DNA damage, and elevated nitric oxide levels, alongside a considerable decline in antioxidant enzyme activity and glutathione (GSH) levels in HANP-exposed rats. However, co-administration of CNPs and/or CUNPs significantly restored neurotransmitter levels, mitochondrial biogenesis, oxidative balance, and inflammation markers. Notably, the combined administration of both CNPs and CUNPs provided superior protection against HANP-induced neurotoxicity compared to individual treatments, suggesting their potential as neuroprotective agents [69]. Qureshi *et al*. formulated risperidone-loaded chitosan lipid nanoparticles (RIS-CH-LNPs) to improve drug efficacy in schizophrenia through intranasal delivery. A three-factor, three-level

optimisation model was used to evaluate its effects on particle size (Y1), drug loading (Y2), and release behaviour (Y3). The optimised formulation (RIS-CH-LNPopt) underwent further characterisation, including surface morphology, *ex vivo* permeation, *in vivo* assessment, and stability testing. The developed RIS-CH-LNPs exhibited a nanoscale size (132.7 nm), high drug loading (7.6%), and extended drug release (80.7%). *Ex vivo* permeation studies demonstrated a 2.32-fold enhancement in drug absorption compared to a conventional risperidone suspension (RIS-SUS). *In vivo* experiments revealed that RIS-CH-LNPopt exhibited significantly greater bioefficacy than RIS-SUS administered via intranasal or intravenous routes. The pharmacokinetic analysis and brain/plasma ratio of RIS-CH-LNPs remained elevated at all time points, confirming direct nose-to-brain drug transport [70]. Table 4 provides a summary of these applications.

Table 4. Chitosan nanosystems in nervous system repair.

Device	Characteristics	Reference
Chitosan nanoparticles	Restoration of nerve impulse transmission	[66]
Chitosan nanoparticles loaded with valproic acid	Functional recovery after injury of spinal cord	[67]
Naringin-loaded chitosan nanoparticles	Alleviation of hyperalgesia and cold allodynia	[68]
Chitosan nanoparticles	Protection against neuroinflammation and oxidation	[69]
Risperidone-loaded chitosan lipid nanoparticles	Improvement of drug bioavailability for the treatment of schizophrenia	[70]

4.5. Drug and Gene Delivery

Chitosan nanoparticles are versatile carriers for delivering pharmaceuticals, genetic material, and bioactive molecules. In regenerative medicine, chitosan-based nanoparticles have been employed to ensure controlled and sustained release of bioactive compounds that support tissue repair. For instance, Mansouri *et al*. engineered folic acid-modified chitosan-DNA nanoparticles to enhance gene delivery by leveraging folic acid-mediated cellular uptake. These nanoparticles were synthesised via reductive amidation and complex coacervation, with the charge ratio (N/P) assessed through laser scattering. DNA loading and integrity were confirmed using gel electrophoresis, and cytotoxicity was evaluated via MTT assay. The nanoparticles measured approximately 118 nm in size, exhibited a positive zeta potential, and maintained 80% cell viability, significantly higher than LipofectAMINE2000-treated samples, which demonstrated only 30% viability. Gel electrophoresis confirmed DNA integrity within the carriers. The folic acid-functionalised nanoparticles exhibited lower cytotoxicity, efficient DNA condensation, and favourable physicochemical attributes, highlighting their potential as a non-viral vector for improved transfection efficiency in gene therapy [71]. De la Fuente *et al*. explored the potential of nanoparticles composed of hyaluronic acid (HA) and chitosan (CS) for gene transfer in ocular applications. These bioadhesive nanoparticles were synthesised using ionotropic gelation and encapsulated plasmids pEGFP and pβ-gal. Evaluations were conducted in human corneal epithelial (HCE) and normal human conjunctival (IOBA-NHC) cells, with confocal microscopy employed to examine nanoparticle internalisation. The particle sizes ranged from 100 to 235 nm, with ζ-potentials varying between −30 and +28 mV.

Transfection studies indicated that HA-CS nanoparticles facilitated effective gene delivery (up to 15% of treated cells) without affecting cell viability. Confocal imaging revealed that uptake was mediated via fluid-phase endocytosis through CD44 receptors [72]. Baghdan *et al*. investigated chitosan's potential as a polycationic, non-viral gene delivery system, focusing on its biocompatibility and biodegradability while addressing its limited transfection efficiency in physiological conditions. To enhance its performance, liposome-encapsulated chitosan nanoparticles (lipochitoplexes, LCPs) were developed. These were created by ionic gelation using low molecular weight, highly deacetylated chitosan cross-linked with tripolyphosphate for plasmid DNA (pDNA) entrapment. LCPs were then formulated by combining chitosan nanoparticles with anionic liposomes (DPPC/Cholesterol), which improved DNA protection, reduced cytotoxicity, and doubled transfection efficiency under physiological conditions. Their effectiveness was validated using the chorioallantoic membrane (CAM) model, where LCPs successfully delivered genes without causing vascular damage [73]. Li *et al*. synthesised a quaternised chitosan derivative, *N*-2-hydroxypropyl trimethyl ammonium chloride chitosan (HACC), and assessed its potential for gene delivery. HACC was prepared using a cationic etherifying agent, with its chemical structure confirmed through Fourier transform infrared (FTIR) spectroscopy and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. HACC-DNA nanocomplexes were formulated via coacervation and characterised for size, charge, and DNA protection ability. Gel electrophoresis and DNase I assays confirmed strong DNA binding and protection. Cytotoxicity tests in mesenchymal stem cells (MSC) demonstrated no adverse effects. Transmission electron microscopy (TEM) revealed that the nanoparticles were circular or oval, with surface charge shifting from negative to positive as the weight ratio increased. The nanoparticles exhibited a biphasic DNA release pattern, with 55% released within 20 hours. The transfection efficiency of HACC/pEGFP-GDNF was comparable to that of liposome/pEGFP-GDNF complexes (33.8% vs. 34%, $P = 0.363$). HACC demonstrated favourable solubility, electropositivity, strong DNA binding, and effective DNA protection, positioning it as a promising non-viral gene vector [74]. Moghadam *et al*. developed chitosan (CH)-chondroitin sulphate (CS) nanoparticles (NPs) as a delivery system for genetic material aimed at addressing the challenges of osteoarthritis (OA) treatment, particularly in achieving cartilage repair. These nanoparticles, composed of two biocompatible polymers, minimise toxicity while enhancing targeted delivery through CS-mediated interactions. The CAG-GFP plasmid was used as a model gene sequence, with successful encapsulation demonstrated. The nanoparticles were characterised by their morphology, size, charge, encapsulation efficiency, and release profile. Comparisons between CH-CS NPs, CH-TPP NPs, and Lipofectamine showed that substituting TPP with CS significantly reduced particle size, while zeta potential remained relatively unchanged. CH-CS NPs exhibited superior plasmid uptake and cell viability. Real-time RT-PCR confirmed effective internalisation of siRNA-loaded nanoparticles and successful knockdown of MMP13 expression in chondrocytes, indicating their potential in gene therapy applications for cartilage repair [75]. The findings from these studies are summarised in Table 5.

Table 5. Chitosan nanosystems in drug and gene delivery.

Device	Characteristics	Reference
Folic acid-chitosan-DNA nanoparticles	Non-viral gene vector candidate for enhanced transfection efficiency in gene therapy applications	[71]
Hyaluronic acid and chitosan nanoparticles	Achieving ocular high transfection efficiency	[72]
Liposome-encapsulated chitosan nanoparticles	Improved pDNA protection, reduced cytotoxicity, and enhanced transfection efficiency	[73]
N-2-hydroxypropyl trimethyl ammonium chloride chitosan and pEGFP-DNA nanocomplex	Excellent solubility, electropositivity, strong DNA binding affinity, and high DNA protection	[74]
Chitosan-chondroitin sulfate nanoparticles	A platform for gene sequence delivery to address the limitations of conventional osteoarthritis treatments	[75]

5. Conclusions and Future Remarks

Nanosized chitosan represents a groundbreaking advancement in regenerative medicine, offering exceptional biocompatibility, bioactivity, and functional versatility, making it an invaluable biomaterial for a wide range of tissue engineering applications. Its ability to enhance cellular interactions, support tissue remodelling, and promote wound healing has been extensively explored in various biomedical fields, including bone regeneration, cardiac repair, skin healing, and nerve regeneration. The unique physicochemical properties of chitosan nanoparticles, such as their high surface area, mucoadhesive nature, and controlled drug release capabilities, make them particularly suitable for targeted therapeutic applications, where precision and sustained action are crucial. Moreover, their capacity to encapsulate and deliver bioactive molecules, including growth factors, drugs, and genetic material, further broadens their potential in advanced regenerative treatments. Despite these promising applications, several critical challenges must be addressed to enable the widespread clinical use of nanosized chitosan. One major hurdle is the scalability and cost-effective production of chitosan nanoparticles with consistent size, stability, and bioactivity [76]. Current synthesis methods often result in batch-to-batch variability, limiting their reproducibility and large-scale manufacturing potential. Developing standardised, high-yield production techniques that maintain the integrity and therapeutic efficacy of the nanoparticles is crucial for their commercial viability [76]. Another significant challenge lies in the precise control of chitosan nanoparticle biodegradation. The degradation rate must be fine-tuned to match the specific requirements of different tissue engineering applications, ensuring that the nanoparticles provide sustained therapeutic benefits without premature breakdown or prolonged persistence, which could lead to undesirable side effects [77]. Advanced modification strategies, such as cross-linking and functionalisation with bioactive molecules, may help achieve better control over degradation kinetics and enhance their performance in targeted

therapies. Furthermore, regulatory and safety concerns remain a major obstacle in translating nanosized chitosan from the laboratory to clinical practice [78]. While preclinical studies have demonstrated promising outcomes, comprehensive *in vivo* studies and large-scale clinical trials are necessary to establish their long-term safety, efficacy, and immunogenic profile. Regulatory approval processes require extensive documentation of toxicity, biocompatibility, and therapeutic effectiveness, necessitating further investment in rigorous research and development [79]. Addressing these challenges will lead to the successful integration of nanosized chitosan into mainstream medical applications, unlocking new possibilities for personalised and precision medicine. With continuous advancements in nanotechnology, biomaterials engineering, and drug delivery systems, nanosized chitosan has the potential to revolutionise regenerative medicine by offering innovative solutions for tissue repair and organ regeneration.

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