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Biopolymer Chitosan: A Review of Modification, Characteristics and Biomedical Activity

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Abstract

Chitosan, a natural cationic biopolymer, is gaining popularity in advanced drug delivery systems (DDS) because to its biodegradability and mucoadhesive characteristics. However, its therapeutic efficacy is frequently hampered by weak solubility at physiological pH and inadequate mechanical stability. This paper gives a thorough overview of the most recent advances (up to 2025) in chitosan functionalization to address these constraints. Scientific articles were collected from online database searches on Science Direct, PubMed, and Google Scholar. Chemical modifications such as synthetic and natural cross-linking (e.g., glutaraldehyde, TPP, genipin, vanillin and tannic acid), carboxylation, alkylation, acylation, Quarternization reaction, Graft Copolymerization and the formation of pH-sensitive Schiff bases have made it possible to develop "smart" stimuli-responsive nanocarriers for targeted cancer and gene therapy. Simultaneously, physical approaches like gamma irradiation provide "green" pathways for producing high-purity, low-molecular-weight chitosan with increased bioactivity. Beyond acting as an inert carrier, modified chitosan demonstrates potent intrinsic antibacterial, antioxidant, anticancer and immunomodulatory activities significantly improving the therapeutic index of encapsulated drugs like paclitaxel and curcumin. This review establishes a roadmap for the clinical translation of multifunctional chitosan derivatives in nanomedicine by bridging the gap between specific modification mechanisms and their biomedical activity.

Keywords: Biomedical activity, Characteristic, Chitosan, Modification

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Kitosan, biopolimer kationik alami, semakin populer dalam sistem penghantaran obat (DDS) canggih karena sifat biodegradabilitas dan mukoadhesifnya. Namun, efektivitas terapeutiknya sering terhambat oleh kelarutan yang lemah pada pH fisiologis dan stabilitas mekanik yang tidak memadai. Makalah ini memberikan tinjauan menyeluruh tentang kemajuan terbaru (hingga 2025) dalam fungsionalisasi kitosan untuk mengatasi kendala tersebut. Artikel ilmiah dikumpulkan dari pencarian basis data daring di Science Direct, PubMed, dan Google Scholar. Modifikasi kimia seperti pengikatan silang sintetis dan alami (misalnya, glutaraldehida, TPP, genipin, vanilin dan asam tanat), karboksilasi, alkilasi, asilasi, reaksi kuaternisasi, kopolimerisasi cangkok dan pembentukan basa Schiff yang sensitif terhadap pH telah memungkinkan pengembangan nanokarier responsif rangsangan "pintar" untuk terapi kanker dan gen yang ditargetkan. Secara bersamaan, pendekatan fisik seperti iradiasi gamma menyediakan jalur "hijau" untuk menghasilkan kitosan dengan kemurnian tinggi, berat molekul rendah dan bioaktivitas yang meningkat. Selain bertindak sebagai pembawa inert, kitosan yang dimodifikasi menunjukkan aktivitas antibakteri, antioksidan, antikanker dan imunomodulator intrinsik yang ampuh secara

signifikan meningkatkan indeks terapeutik obat-obatan yang dikapsulasi seperti paclitaxel dan curcumin. Tinjauan ini menetapkan peta jalan untuk translasi klinis turunan kitosan multifungsi dalam nanomedisin dengan menjembatani perbedaan antara mekanisme modifikasi spesifik dan kemanjuran biologisnya.

Kata Kunci: Aktivitas biomedis, Karakteristik, Kitosan, Modifikasi

INTRODUCTION

Natural biopolymers have emerged as the preferred biomaterials in the pharmaceutical business due to their inherent biodegradability, non-toxicity, and ecological sustainability. Chitosan, a linear polysaccharide made up of beta-(1→4)-linked D-glucosamine and N-acetyl-D-glucosamine, candidate for enhanced drug delivery systems (DDS) [1]. Chitosan, which is predominantly derived from chitin deacetylation, has unique cationic characteristics that promote mucoadhesion and controlled release. However, the therapeutic application of natural chitosan is frequently hampered by its low solubility at physiological pH, fast enzymatic breakdown, and inferior mechanical durability in acidic conditions [2]. To handle these structural limitations, researchers have focused on functionalizing chitosan via several modification mechanisms.

Chemical modification, relying on the chitosan backbone's reactive amino (-NH_2) and hydroxyl (-OH) groups [3]. Cross-linking with synthetic agents like glutaraldehyde and TPP as well as natural alternatives like genipin and vanillin has been demonstrated to significantly affect drug release kinetics and gastroretentive characteristics [4]. Furthermore, advanced processes such as carboxylation, acylation, quaternization, and the production of Schiff bases enable the development of "smart" nanocarriers with stimuli-responsive behaviour which is particularly useful for targeted cancer therapy and gene transfer [2]. In addition to chemical treatments, physical alterations such as gamma irradiation and ultrasonic treatment provide "green" options. These approaches enable careful control of molecular weight and crystallinity without the use of potentially hazardous chemicals, yielding Low Molecular Weight Chitosan (LMWC) with improved biological solubility and purity [5].

Chitosan's functionalization not only improves its role as an inert carrier but it also greatly increases its intrinsic biological activity. Recent research indicates that modified chitosan derivatives have powerful antibacterial, antioxidant, anticancer, and immunomodulatory properties. For example, while native chitosan has low antibacterial activity, its thiolated or sulfonated derivatives have a greater ability to eliminate biofilm-embedded microorganisms and suppress tumor development [6]. Although the literature on chitosan modification has expanded rapidly, most existing reviews remain fragmented, focusing narrowly on either conventional chemical modifications or physical techniques in isolation. This review article provides a comprehensive and up-to-date assessment of recent advances through 2025. The current state of the art focuses not only on enhancing chitosan solubility at physiological pH via carboxylation or acylation but also on developing stimuli-responsive, smart drug delivery systems through grafting and Schiff base formation for precise antitumor targeting. Furthermore, the integration of physical methodologies, such as gamma irradiation, currently enables finer control over nanovesicle size, thereby enhancing the bioavailability of critical therapeutics. Moving beyond a single modification paradigm, this article elucidates how physical modification (irradiation) can complement or even supplant chemical modification within the framework of Green Chemistry. Finally, it integrates diverse chitosan modification strategies to optimize the therapeutic index of poorly soluble drugs, such as paclitaxel and curcumin, thereby offering a strategic roadmap for future clinical applications in the field of nanomedicine.

METHODS

A scientific literature search was conducted using papers from internet databases such as Science Direct, PubMed, ResearchGate, and Google Scholar. The database search options include the years 2015 through 2025. Research papers prepared in English and published in peer-reviewed journals were eligible for inclusion. Exclusion criteria included articles that lacked unambiguous experimental data, case and conference proceedings that did not match inclusion criteria. 52 publications were examined and categorized based on modification methods, characteristics chemical modification biopolymer of chitosan and biomedical activity of chitosan and derivatives.

RESULTS AND DISCUSSION

Chemical Modification Biopolymer of Chitosan

Crosslinked synthetic and natural

Modification of chitosan through cross-linking aims to improve mechanical stability, regulate drug release rates, and reduce the hydrophilicity of chitosan

so that it does not dissolve easily in acidic media. In general, cross-linking compounds are divided into two categories: synthetic (effective but often toxic) and natural (safer/biocompatible) Chitosan has been cross-linked synthetic with other chemical agents such as glutaraldehyde, epichlorohydrin (ECH), ethylene glycol diglycidyl ether (EDGE) and sodium tripolyphosphate (Na TPP) which are generally being used to improve the chitosan hydrogels used in water treatment and cross-linked natural with Genipin, Vanilin, Citric Acid and Tannic Acid [4,7].

Crosslinked synthetic chitosan-glutaraldehyde

The terminal carbonyl groups (-CHO) of glutaraldehyde crosslink with the primary amine groups (-NH_2) of chitosan, yielding stable carbon-nitrogen double bonds (C=N, imine linkages) via condensation. To optimize processability, natural chitosan (CS) was blended with synthetic polyethylene oxide (PEO) at an optimal 8:2 weight ratio and fabricated into nanofibrous matrices using electrospinning. Prior to electrospinning, the hydrophobic antacid drug Nizatidine (NIZ) was incorporated directly into the polymer blend. Within this matrix, the ether oxygen atoms of PEO act as hydrogen-bond acceptors that interact with the protonated amine groups of CS [8]. This combination of covalent crosslinking and interchain hydrogen bonding restricts polymer chain mobility, resulting in a rigid, partially hydrophobic three-dimensional matrix. In a structurally analogous approach, CS was blended with polyvinyl alcohol (PVA) via electrospinning and loaded with gallic acid (GA) to serve as a model drug and antioxidant agent. To overcome the inherent water solubility and mechanical instability of the as-spun PVA-CS-GA fibers, post-spinning chemical crosslinking was performed using glutaraldehyde in either the vapor or liquid phase. This treatment promotes covalent crosslinking between the polymer networks, while the hydroxyl groups (-OH) of PVA and GA form robust intermolecular hydrogen bonds within the matrix. Consequently, these synergistic interactions yield a highly stable and consolidated three-dimensional network structure [9].

Crosslinked synthetic chitosan-tripolyphospate (TPP)

TPP modification, as opposed to glutaraldehyde, relies on electrostatic (ionic) interactions rather than irreversible covalent bonds. The number of free amine groups is an important element for accurately controlling this change. The polyvinyl sulfate potassium (PVSK) titration method has been established as an accurate technique for monitoring the decrease in free amine groups as the concentration of the crosslinking agent increases, as well as a direct indicator for measuring the degree of crosslinking in both covalent and ionic systems [7]. Ionic cross-linking utilizing food-grade natural or synthetic polyanions such as Sodium Tripolyphosphate (STPP/TPP) is gaining popularity as a safer option due to its non-toxic, edible nature, and reversible interactions. The number of free amine groups NH_2 is a vital metric for monitoring and precisely controlling the modification reaction [10].

Crosslinked synthetic chitosan-dialdehyde

Dialdehyde chitosan (DACS) is produced rapidly and efficiently (usually by a periodate oxidation process of chitosan to convert glycol groups to dialdehyde groups) (see **Figure 1**) [11]. The generated DACS is then used in varying amounts or concentrations to create a new material in the form of a chitosan film.

The crosslinking mechanism between chitosan and aldehyde compounds occurs via a covalent condensation reaction that forms permanent covalent bonds. This process begins with a nucleophilic attack by a free primary amine group (-NH_2) on the chitosan chain toward the aldehyde carbonyl group (-CH=O). This reaction then releases a water molecule (H_2O) and produces an imine bond (-C=N-). When the aldehyde compound used is bifunctional or polyfunctional (such as glutaraldehyde or other dialdehydes), the aldehyde groups at both ends of the molecule will bind to the amine groups of two different chitosan chains. It is these interchain covalent bridges that create a three-dimensional crosslinked network, which significantly enhances the thermal stability, mechanical strength and resistance of the chitosan material to degradation in acidic media. Modifications were systematically carried out to transform the basic properties of pure chitosan, which is generally insoluble into a water-soluble, biocompatible polymer. The steps involved include, Thermal organic acid hydrolysis (to break the polymer chains into shorter segments), periodate oxidation (to break glycolic bonds and form dialdehyde functional groups), precipitation (to purify the final Dialdehyde chitosan product) [12].

Crosslinked Synthetic Chitosan - Epichlorohydrin (ECH)

The chemical modification of natural chitosan (CS) to synthesize novel derivatives typically proceeds through a multi-stage framework (**Figure 2**). This begins with Schiff base formation driven by the nucleophilic attack of primary amine groups (-NH_2) on carbonyl carbons to yield imine bonds (-C=N-) followed by Epichlorohydrin-mediated crosslinking

via epoxide ring-opening or nucleophilic substitution, and culminates in amine grafting to introduce new active centres

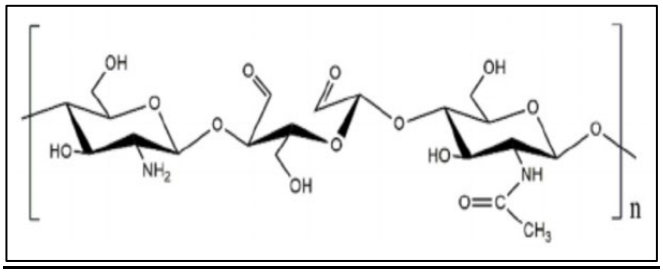

Figure 1. chemical structure of dialdehyde chitosan (DACS) [11]

This synergistic modification effectively disrupts the inherent crystallinity of CS, expands intermolecular spacing, elevates swelling capacity, and alters solubility profile [13]. Correspondingly, Dumont *et al*. utilized ECH to chemically crosslink organic - inorganic bio nanocomposites composed of either pure CS or its hydrophilic derivative, carboxymethyl-chitosan (CMC), embedded with hydroxyapatite nanoparticles (nHA). In this system, ECH covalently bridges the active hydroxyl (-OH) amine ($-NH_2$) or carboxymethyl functionalities across the polymer chains, yields a structurally stable membrane, and uniquely modulates the nucleation and crystal growth kinetics of the intra-matrix nHA [14]. Both approaches underscore the identical mechanistic role of ECH in transforming linear biopolymers into robust, functionalized three-dimensional networks [14].

Crosslinked synthetic chitosan-ethylene glycol diglycidyl ether (EGDE)

The mechanism of crosslinking between chitosan (CS) and EGDE is highly dependent on the reaction conditions, particularly pH, acid type, and polymerization temperature. Unlike alkaline media that target amine groups, reactions under acidic conditions (using HCl) force EGDE to bind specifically to the hydroxyl group (-OH) at the C6 position of the chitosan glucosamine unit, resulting in a gel structure that is mechanically much more stable than when using acetic acid. In addition to acidity, temperature control also plays a crucial role. At room temperature, this reaction produces a combination of covalent cross-links and side-chain functionalization capable of physically trapping drug molecules such as amoxicillin within its matrix. Meanwhile, at subzero temperatures (-10°C), a phenomenon known as cryo-concentration occurs, where the freezing of the solvent forces the chitosan and EGDE to accumulate in the unfrozen liquid phase, thereby facilitating the formation of effective and robust cryogel cross-links despite a lower degree of chemical modification [15,16].

Crosslinked natural chitosan-genipin

The synthesis mechanism of modified chitosan films involves the chemical formation of covalent cross-links between the free amine groups on the chitosan chains and genipin, which acts as a natural cross-linking agent. Upon mixing with components such as polyvinyl alcohol (PVA) and active agents (curcumin or astaxanthin), genipin specifically targets the chitosan amine groups to form a robust interpenetrating network. The success of this covalent cross-linking reaction is demonstrated spectroscopically through shifts in wavenumbers in the FT-IR spectrum (such as the appearance or shift of peaks in the 1720cm^{-1} and 1569 cm^{-1} regions), indicating that the functional structure of genipin has successfully bridged the chitosan polymer chains into a continuously integrated film matrix. The curcumin-loaded genipin-chitosan- polyvinyl alcohol films were biocompatible. To promote wound healing, curcumin was also added to the genipin-chitosan- polyvinyl alcohol formulation. These films also mediated faster healing in wounds created on albino Wistar rats, confirming their potential as wound dressings [17]. In study Patthrare *et al*., genipin-crosslinking is not only a promising strategy for improving the performance and application of develop chitosan-astaxanthin film but it is also a natural crosslinker and an alternative to most chemical crosslinkers which induce unwanted alterations and cytotoxicity [18].

Crosslinked natural chitosan-vanillin

The synthesis mechanism of this 3D macro-hydrogel involves the formation of covalent crosslinks between chitosan (CS) and vanillin, which acts as a non-toxic natural crosslinking agent. The main reaction occurs through the formation of Schiff base bonds resulting from the coupling between the primary amine groups (-NH2) on the chitosan chains and the aldehyde groups (-CHO) on the vanillin molecules. In addition to these covalent Schiff base bonds, the stability of this three-dimensional interpenetrating network is further reinforced by the formation of hydrogen bonds that are extensively distributed throughout the hydrogel matrix. The success of this covalent crosslinking reaction and these intermolecular interactions was confirmed spectroscopically using Fourier Transform Infrared Spectroscopy (FTIR)[19].

Crosslinked natural chitosan-tannic acid

The synthesis mechanism of tannic acid-loaded chitosan nanoparticles (CS-TA-NPs) was carried out using the ionic gelation method. In this process, tannic acid (TA) acts as an active polyanionic agent that undergoes electrostatic cross-linking (ionic cross-linking) with the positively charged primary amine groups on the cationic chitosan chains. This ionic cross-linking reaction and the entrapment of the active compound occur spontaneously in the liquid phase, forming a physically stable polyelectrolyte complex [20].

Carboxylation

Chemical carboxylation modification of the chitosan biopolymer introduces carboxylic acid functional groups (-COOH) into the polymer backbone via distinct reaction pathways depending on the specific modifying This modification adds a carboxyl group (-COOH) to the chitosan chain. Monochloroacetic acid is often used under basic conditions. Increases solubility in air across a wide pH range (amphoteric) and enhances metal ion or cationic drug binding. N-succinyl chitosan (NSC) was produced with succinic anhydride in a ring-opening reaction under heterogeneous circumstances. The research results show that altering the degree of replacement of NSC has a considerable impact on essential hydrogel characteristics. The research also adds to the development of hydrogels for next-generation wound care applications [21]. The research Ibrahim *et al*., N-carboxyethylchitosan (CECS) was successfully synthesized using the Michael addition reaction of chitosan (CS) with acrylic acid in water. [22]. The study Olanipekun *et al*., focuses on the production and antibacterial properties of chitosan (CS), carboxymethyl chitosan (CMC) and their metal composites. The success of all these covalent carboxylation reactions is spectroscopically validated through ^{1}H-NMR and FTIR analyses which exhibit distinct shifts and the emergence of new functional peaks indicating the successful incorporation of the carboxyl groups [23].

Alkylation

Alkylation involves the addition of alkyl groups to the nucleophilic nitrogen (amine) or oxygen (hydroxyl) positions of the chitosan backbone. This reaction typically occurs between chitosan and alkyl halides leading to a significant disruption of the polymer's inherent crystalline structure. Consequently, the resulting alkylated chitosan exhibits enhanced water solubility over a wider pH range, eliminating the need for acidic solvents. [24].

Acylation

Chitosan's amine group reacts with carboxylic acid derivatives. Types include N-acylation (on nitrogen) and O-acylation (on oxygen). Drug Delivery Purpose, reduce crystallinity while increasing hydrophobic characteristics. Acylated chitosan frequently produces self-assembled micelles, which are excellent for encapsulating hydrophobic medicines (poorly soluble in water), such as Paclitaxel [25]. Acylated chitosan (Myristoyl and Octanoyl) coated paclitaxel-loaded liposomal formulations were produced to alleviate cremophor EL-related toxicity. They were tested for drug entrapment in vitro drug release, cytotoxicity and cell uptake behavior with A549 cells. The myristoyl chitosan-coated liposomal system (LMC) outperformed other formulations in terms of pharmacokinetics, biodistribution and tumor uptake. These findings supported the potential use of acylated chitosan coated liposomal delivery systems (LMC) for tumor targeting with paclitaxel and other medicines.

Quarternization reaction

The process of replacing hydrogen atoms in amine groups with alkyl groups to produce quaternary ammonium salts (R_4N^+). Popular examples include N,N,N-trimethyl chitosan (TMC). Maintains a constant positive charge regardless of surrounding pH levels. Specific applications, very important in gene delivery because the strong positive charge can connect to negatively charged DNA/RNA via electrostatic interactions [26]. In this study [27], created stable reduced graphene oxide (rGO) solutions using the renewable resource quaternized chitosan (QCS) and layered silicate as green reducing and stabilizing agents. The rGO/QCS/layered silicate composite could be built through electrostatic contact and cation exchange. The inclusion of layered silicate increased the composite's thermal stability, and rGO/QCS/OREC demonstrated substantial absorption (425 μg/mg) toward double-stranded DNA.

Graft copolymerization

Chemical modification via graft copolymerization introduces new polymer chains onto the chitosan backbone, enhancing its inherent properties and expanding its functionalities. Generally synthesized via free radical, radiation, or enzymatic pathways [28], free radical initiation in aqueous media remains the most widely utilized method. For instance, temperature-responsive chitosan-graft-poly(N-

isopropylacrylamide) (CS-g-PNIPAAm) has been synthesized through free-radical polymerization of chitosan and N-isopropylacrylamide (NIPAAm) monomers using potassium persulfate (KPS) as the initiator. In this mechanism, the chitosan-to-monomer molar ratio (ranging from 1:0.25 to 1:10) can be systematically adjusted to control the density and length of the grafted side chains within the chitosan matrix [29].

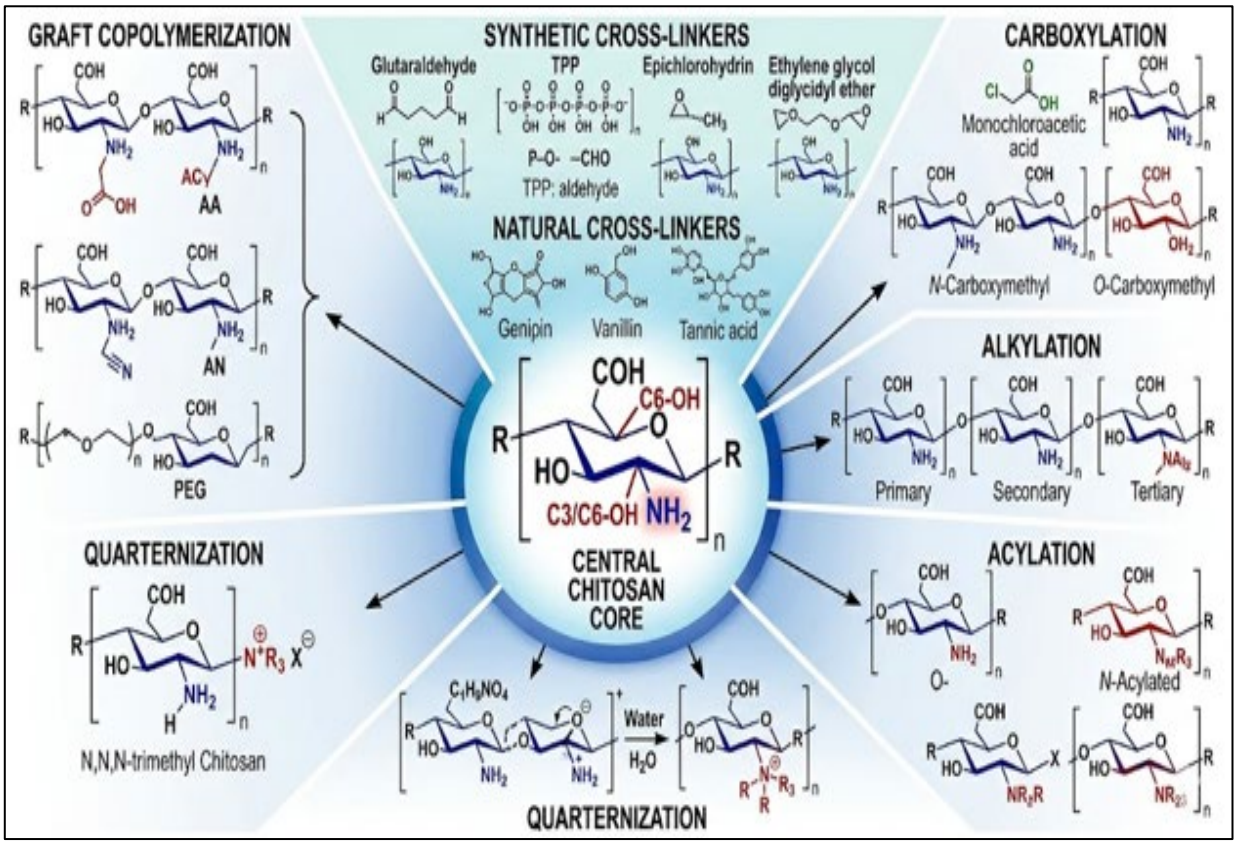

Figure 2. Integrated pathway for chemical modification of chitosan biopolymer

Schiff base

The condensation reaction between chitosan's primary amine group and the carbonyl group (aldehyde or ketone) produces an imine bond (-C=N-). This bond is pH-sensitive; it is stable at alkaline pH but easily hydrolyzes (breaks) in acidic circumstances. Highly effective for tumor targeting because the cancer environment is acidic the Schiff base bond will dissolve at the cancer site, resulting in precise medication release [30]

PHYSICAL RADIATION MODIFICATION BIOPOLYMER OF CHITOSAN

Physical modification of chitosan is frequently regarded as a "greener" or safer strategy since it avoids the production of new covalent bonds via complex chemical reactions. Radiation modification makes use of high-energy sources such as gamma rays (gamma), electron beams, and ultraviolet (UV) radiation. High-energy radiation causes the breaking of glycosidic linkages (beta-1,4) in the chitosan polymer chain, resulting in the production of free radicals. Furthermore, radiation techniques are more environmentally benign, producing less chemical waste and reducing reliance on harmful compounds [5]. The absence of extra chemicals not only decreases the possibility of contamination but also aligns with green chemistry principles making this technique more ecologically benign than traditional synthesis methods [35].

CHARACTERISTICS AND APPLICATIONS OF CHEMICALLY MODIFIED CHITOSAN

Crosslinked Modification

Cross-linking transforms linear chitosan into robust three-dimensional networks, which are broadly divided into synthetic and natural categories

Synthetic Cross-linking Agents

Glutaraldehyde & Dialdehydes: React via covalent condensation between terminal carbonyl groups (-CHO) and primary amines (-NH2) of CS to

form stable imine bonds (C=N). Characterized by FTIR stretching vibrations (e.g.,-CHO at 1744cm-1), morphology bead-free nanofibers (SEM). Synthetic crosslinking agents glutaraldehyde, characterization morphologically the resulting nanofibers exhibit a continuous, homogeneous and bead-free structure with a fine average diameter of approximately 119.17+22.05nm which contributes to a high mucoadhesive capacity of 22.82+3.21g/cm2, swollen mat-like form (swelling = 29.47 + 3.50% at 24 hours) in acidic environments (pH 1.2) and cytocompatibility assays on Caco-2 cells demonstrate a cell viability of > 86.87 + 6.86%. Applications include controlled drug delivery (e.g., Nizatidine), improved thermal/mechanical stability and antimicrobial films [8,9]. Characterization tests validated the successful synthesis of dialdehyde chitosan (DACS) with an 82% aldehyde content. This modification achieved an 89% increase in solubility and demonstrated superior bioactivity over pure chitosan, exhibiting strong antimicrobial properties and enhanced antioxidant capacity, with DPPH and ABTS radical scavenging efficiencies rising to 97.4% and 31.1%, respectively [11,12].

Sodium Tripolyphosphate (TPP/STPP): Relies on reversible ionic/electrostatic interactions between the positive charge of CS ammonium ions ($-NH_3^+$) and negative polyanions. Characterized FTIR, shift in absorption spectra of −OH and $-NH_2$ groups due to polyelectrolyte complexation with phosphate bonds, XRD disruption of crystalline structure into a random amorphous phase, high colloidal stability (zeta potential > +30mV), morphology (SEM, TEM), Entrapment Efficiency, drug loading capacity (DLC). In study Wawaimuli *et al*., The particle size of the curcumin nanoparticles obtained were 11.5 nm, entrapment efficiency (EE) of curcumin nanoparticles were exceeding 99.97%, and drug loading capacity (DLC) was 11.34% [36].The average particle size of chitosan-TPP nanoparticles had molecular weights of 97.3 kDa, 62.2 kDa, and 40.9 kDa, all within the range of 150 nm, chitosan nanoparticles with low molecular weight have strong antibacterial qualities [7]. Used extensively for safe, non-toxic and edible nanoparticle formulations for safe drug delivery.

Epichlorohydrin (ECH): Proceeds through epoxide ring-opening or nucleophilic substitution to covalently bridge polymer chains. Characterized by decreased crystallinity and increased amorphous properties via XRD, in vitro/in vivo up to 55% improvement in bone repair compared to controls. Applied in antibacterial materials tests showed moderate to high antibacterial activity against *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) [13] and bone tissue regeneration membranes [14].

Ethylene glycol diglycidyl ether (EDGE): A comprehensive characterization of chitosan/EGDE (CS/EGDE) hydrogels and cryogels successfully revealed their unique chemical, structural, mechanical and viscoelastic properties. Advanced spectroscopic analyses, including FT-IR, elemental analysis and solid-state ^{13}C and ^{15}N NMR. Morphologically, the cryogelation process was shown to produce a highly porous super macroporous structure that directly correlates with superior mechanical strength, as evidenced by Young's modulus values reaching up to 90 kPa. Furthermore, rheological testing revealed that chitosan concentration plays a key role in determining the hydrogel matrix density, while exposure to ultrasound waves (43 kHz) triggers a viscoelastic response in the form of matrix softening. This softening effect varies depending on the chitosan concentration, the most significant changes were observed at low concentrations (1.5% and 2%) whereas at higher concentrations (2.5% and 3%) the dense structure limits the degree of softening although the effect remains detectable. Used for physical drug entrapment (e.g., Amoxicillin) [15,16].

Natural Cross-linking Agents

Genipin: Covalently links free amine groups to form a robust interpenetrating network. Characterized by distinct FTIR peak shifts at 1720cm^{-1} and 1569 cm^{-1}, SEM, rougher surface structure but retains uniform homogeneity, mechanical up to a 192% increase in tensile strength [17], the optimal genipin concentration (such as at 1% w/w) successfully improves the film's barrier properties, as evidenced by increased water retention capacity and a reduced water vapor transmission rate (WVTR) while the oxygen transmission rate (OTR) shows only a slight increase. Applied as safe, non-toxic wound dressings e.g., loaded with curcumin [17] or astaxanthin [18].

Vanillin: Forms covalent Schiff base bonds alongside widespread intermolecular hydrogen bonding. Characterized by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (FE-SEM) as an interconnected 3D pore structure with >90% porosity. Applied as macro-hydrogels with superior mechanical strength when swollen, The Chitosan-Vanilin hydrogel exhibited good antibacterial activity and cell viability [19].

Tannic Acid: Characterized via FTIR and XRD to confirm polyelectrolyte complexation. In study Marzieh *et al*., it was loaded into chitosan-based

nanoparticles (CS-NPs) to overcome these limitations and enhance its antibacterial and anticancer properties. TA-loaded CS-NPs (CS-TA-NPs) were produced using ionic gelation and physicochemically studied using FE-SEM, FTIR, XRD, PDI, DLS and zeta potential analysis. The antibacterial, cytotoxic and epigenetic effects of CS-TA-NPs were stronger than those of free TA and unloaded CSNPs [20]. Used as nano-carriers to enhance the bioavailability, antibacterial and anticancer efficacy of anti-tumor drugs.

Functional Group Substitutions & Derivations

Carboxylation: Characterized by NMR & FTIR: Spectroscopically validated through distinct shifts and emergence of new functional peaks (^{1}H-NMR and FTIR). Drastic decrease in native CS crystalline regions to form an amorphous polymer (XRD), X-ray diffraction (XRD), thermogravimetric analysis (TGA) and Differential scanning calorimetry (DSC), porous hydrogel structure where pore size is proportional to the degree of substitution (SEM), swelling capacity, equilibrium water retention and water vapor transmission rate (WVTR). It yields amphoteric polymers with excellent water solubility across a wide pH range , optimized for next-generation wound care [21], antibacterial and enhanced drug/metal ion binding [22,23].

Alkylation: Adds alkyl groups to the nucleophilic nitrogen or oxygen positions via alkyl halides. Characterized by disrupted crystalline structures and enhanced water solubility without acidic solvents. Successfully applied as micro channelled alkylated chitosan sponges (MACS) featuring rapid shape-memory behavior for massive hemorrhage control and clinical translational potential in treating deadly noncompressible bleeding and promoting wound healing [24]

Acylation (N-acylation or O-acylation): Reacts CS amine groups with carboxylic acid derivatives to enhance hydrophobic traits and reduce crystallinity. Characterized by zeta potential, shift in surface charge from negative (−10.5 mV) to positive (+21 to +27 mV) upon coating, homogeneous average particle size in the range of 180–200 nm by sizing. Applied to create self-assembled micelles or liposomal coatings for encapsulating poorly soluble drugs (e.g., Paclitaxel) for targeted tumor delivery [25]

Quaternization: Replaces hydrogen atoms in amine groups with alkyl groups to form quaternary ammonium salts (e.g., N,N,N-trimethyl chitosan/TMC). Characterized by TEM, AFM and XRD analyses confirmed the successful loading of rGO nanosheets with layered silicates specifically rectorite (REC) and organic rectorite (OREC) via a cation exchange mechanism, forming rGO/QCS/layered silicate composites. The rGO/QCS/OREC composite exhibited a higher zeta potential (+38.5 mV) than its REC variant (+37.3mV), indicating a superior stabilizing effect. Crucial for gene delivery systems to bind negatively charged DNA/RNA via electrostatic interactions [26,27].

Schiff Base Formation: A Schiff base of Chitosan was created by condensing Chitosan (CS) with six aromatic aldehydes and validated by FT-IR, NMR, XRD, TGA and DSC. Schiff bases were examined for antibacterial activity against Gram-positive/negative bacteria and fungus as well as anticancer activity against cell lines MCF-7, HCT-116 and HepG2. The results research Alamri *et al*. suggest that Schiff bases have stronger antibacterial activity than amoxycillin and tetracycline but no cytotoxic action when compared to colchicine. This modification adds a carboxyl group (-COOH) to the chitosan chain. Highly effective for precise tumor-targeted drug release within acidic cancer microenvironments, showing potent antimicrobial and anticancer activity [30]

Graft Copolymerization

Covalent side-chain grafting is quantitatively validated using ^{1}H-NMR and FTIR. Application, imparts unique stimuli-responsive traits, such as thermoresponsive phase separation at a Lower Critical Solution Temperature (LCST of 29.0–32.7°C) when grafted with poly(N-isopropylacrylamide) (PNIPAAm). It significantly enhances heavy metal adsorption, biodegradation and wound-healing efficiency [28,29].

Physical Radiation (gamma irradiation technique)

Physical alterations, such as gamma irradiation can alter chitosan's surface characteristics, drug release rate, and stability. Gamma irradiation can improve medication release and minimize edema without creating significant structural changes in chitosan microparticles. Characterized by FTIR, GPC, significant decrease in molecular weight without altering the degree of deacetylation (DD). Advantages for drug delivery, this process produces Low Molecular Weight Chitosan (LMWC) or chitosan oligomers with significantly higher water solubility and bioactivity (such as antioxidants and antitumor) while leaving no chemical residues. Exploring physical alterations such as gamma irradiation and nanoparticle integration to improve drug release, bioavailability and

tumor targeting while overcoming the limits of curcumin's low solubility and bioavailability. Zhao *et al*. investigated the combination of natural and synthetic polymers hyaluronic acid (HA) / chondroitin sulfate (CS) / polyvinyl alcohol (PVA) for the release of cefazoline and theophylline at dosages ranging from 5 to 25 kGy [31]. Taşdelen *et al*. (2018) used gamma irradiation at a dosage of 25 kGy to liberate 5 Florouracyl from CS/HA/HAP hydrogels [32]. Sabaghi *et al*. created chitosan nanoparticles with catechin mixed with CS/PVA and crosslinked at doses of 0 kGy, 40 kGy, and 60 kGy for catechin drug release in low-fat and high-fat simulants and studied release modelling [33]. The incorporation of irradiation silver nanoparticles into chitosan-coated vesicles improves mucoadhesion (ability to stick to mucosal layers), bioavailability, and cytotoxicity against cancer cells, owing to enhanced reactive oxygen species and radio sensitization [34]. One of the most notable benefits of adopting radiation technology for synthesis is the exceptional purity of the produced nanoparticles, as this method frequently removes the need for additional chemical reagents, lowering the chance of contamination.

BIOMEDICAL ACTIVITY OF CHITOSAN AND DERIVATIVES

Antibacteria

Chitosan possesses intriguing antibacterial action, good biodegradation, high biocompatibility, low toxicity, and great physical and chemical properties. As a result, chitosan is frequently employed in the field of antibacterials. Chitosan and its derivatives as seen on **Figure 3**, demonstrate antibacterial action against fungi, gram-positive and gram-negative bacteria [37]. Pelegrino *et al*., report a simple and effective approach for chemically modifying chitosan to produce S-nitroso-chitosan for antibacterial applications. Lower minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values suggest that S-nitroso-CS has more antibacterial activity than chitosan (CS) and thiolated chitosan (TCS). Time-kill curves revealed that after 0.5 hours of incubation with S-nitroso-CS, bacterial cell viability dropped 5-fold for *E. coli* and 2-fold for S. mutans compared to their respective controls. The CS backbone was chemically changed with S-nitroso moieties, resulting in a polymer capable of producing therapeutic nitric oxide concentrations with a high antibacterial impact [38]. Based on the experimental results [39]. it is possible to conclude that alkylsulfonation of chitosan might greatly improve efficacy in killing biofilm-embedded bacteria, as well as inhibiting actions against biofilm formation of *E. coli* and *S. aureus*. The use of sulfonated chitosan (SCS) is useful in expanding our understanding of an alternative to antibiotics and chemical preservatives in the food and medicinal areas. Muley *et al*., report that normal and irradiated chitosan have minimal inhibitory concentrations of 2500 and 2000 mg/L for Alternaria spp. and 1750 and 1500 mg/L for Fusarium spp. Normal and irradiated chitosan had IC_{50} values of 1387.9 ± 9.2 and 954.3 ± 6.1 mg/L for Fusarium spp. and 1536.1 ± 24.3 and 1416.8 ± 3.5 mg/L for Alternaria spp [40].

Antioxidant

Oxidative stress is a leading cause of numerous diseases, including cancer, immunological damage, and cardiovascular disease. Free radicals can produce oxidative reactions, disrupt cell structure and function, and harm organ tissues. Chitosan and its derivatives have been shown to be good antioxidants [41,42]. In the research Qing Li *et al.* [43], monophenol and ortho-diphenol were incorporated into chitosan via chemical modification to produce chitosan derivatives with strong antioxidant activity. In vitro investigations showed that the chitosan derivatives were biocompatible and had a high antioxidant capability for reducing oxidative stress. The functionalization of chitosan with phenolic groups enables potential uses in the treatment of oxidative illnesses. The study investigated the influence of gamma irradiation-induced molecular weight (MW) reduction on the antioxidant capacity of chitosan, with the potential for food preservation applications. Chitosan was irradiated using a Cobalt 60 source at dosage rates of 5, 10, 20, and 50 kGy per hour. The antioxidant capability of the chitosan solutions increased as the MW of chitosan decreased due to gamma irradiation [44].

Anticancer

In vitro and in vivo studies have shown that curcumin-loaded chitosan nanocarriers induce apoptosis, inhibit proliferation (growth), and reduce migration/invasion in various cancer cell lines (glioma, colon, breast, pancreatic, ovarian, and head/neck cancer) In U-87MG2 glioma cells, the sodium alginate (SA), chitosan (CS) and cerium oxide (CeO_2)@CUR nanoparticles exhibited the strongest inhibitory effect compared to Free CUR. Cancer cell viability decreased drastically to 45% at 72 hours, while free curcumin was only able to suppress viability to 50–60%. This increase in efficacy is driven by the induction of oxidative stress. The nanoparticle formulation triggered a 75% increase in the production of Reactive

Oxygen Species (ROS) in cancer cells, which is significantly higher than in control cells or other treatments. The use of the CS/SA/CeO_2 hybrid carrier successfully reduced the IC50 value in target (cancer) cells through increased intracellular ROS generation, while simultaneously minimizing toxicity to normal neural tissue [45].

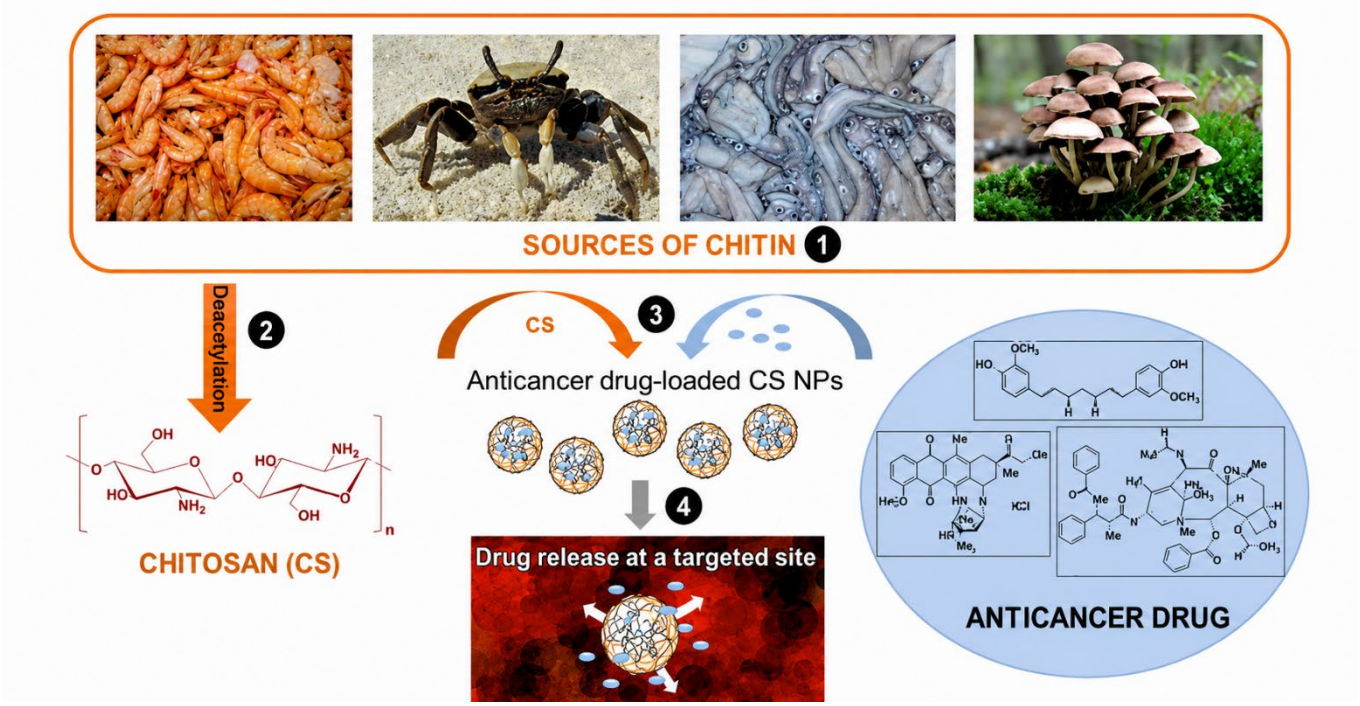

Figure 3. Schematic pathway from natural products to efficient transport and release of anticancer medicines to targeted areas using chitosan-based nanoparticles [46]

The study demonstrated that curcumin-loaded nanoparticles (NPs) exhibited significantly higher anticancer efficacy compared to free curcumin against human colon adenocarcinoma HT-29 cells. While specific IC50 numerical values were not explicitly detailed in the summary, the potency was evidenced by a substantial 83% reduction in cell proliferation for curcumin-loaded NPs, which far outperformed the 50% reduction achieved by free curcumin at equivalent concentrations. This indicates that entrapping curcumin within the Eudragit RS NP-loaded chitosan/HPMC microcapsule system enhances its cytotoxicity and therapeutic potential for targeted colorectal cancer treatment.[47]. Recent advancements in chitosan-based Lipid-Polymer Hybrid Nanoparticles (LPHNPs) have demonstrated significant potential for co-delivery systems. Research indicates that optimized nanoparticles (approximately 225 nm) with a cationic surface charge can achieve encapsulation efficiencies exceeding 80%. Such systems exhibit a synchronized, controlled release of dual agents, such as curcumin and cisplatin, without a burst release effect. Notably, in vitro evaluations using A2780 ovarian cell lines and 3D tumor spheroids have shown that these chitosan-modified nanocarriers enhance chemosensitization and therapeutic efficacy, mimicking in vivo conditions more accurately than traditional 2D models [48]. The co-delivery system (curcumin with other drugs such as cisplatin, doxorubicin, or colchicine) increases cytotoxicity (cell toxicity), overcomes drug resistance, and provides synergistic anticancer effects (stronger combined action) [48,49] and as a Selective toxicity, this chitosan Nanocarrier shows minimal toxicity to normal cells thereby increasing the therapeutic index [50,51]. Curcumin (CUR) - Cyclodextrin (CD) – Chitosan (CS) nanoparticles formulations in terms of cytotoxicity in the A549 cell line and antioxidant activity and release behaviour of curcumin-loaded CDs-CS were examined. Activity of Pure Curcumin (CUR) has a value of approximately 95.12 AU (Radical-Scavenging Activity). Interestingly when formulated as a CUR/β-CD, the value is nearly the same (95.15 AU). Effect of Chitosan (CS) addition, the antioxidant activity actually decreases (to the 50s) compared to without chitosan. This may be because the chitosan layer encapsulates the curcumin, thereby slightly hindering direct interaction with DPPH radicals. Results of anticancer testing using the MTT

assay on human A549 lung cancer cells were incubated with three different concentrations (15, 30 and 45 μg/mL) of the compounds. CUR, CUR/β-CD, CUR/γ-CD, CUR/β-CD-CS and CUR/γ -CD-CS showed an increase in cytotoxicity from 116% for CUR to 85% for CUR/β-CD-CS and 61% for CUR/γ-CD-CS at the optimal and lowest concentration of 15 μg/mL against A549 cells. CUR/β-CD-CS and CUR/γ-CD-CS exhibited cytotoxicity against A549 cells, indicating the biocompatibility of these materials following the binding of CS nanoparticles to β-CD. The IC50 value for CUR/γ-CDCUR/CD-CS was calculated from their inhibitory percent-concentration curves using the DPPH test. A quantified ~43% reduction in instantaneous radical scavenging capacity (decreasing from 95 AU to approximately 54 AU) within the DPPH assay directly corresponds to a 47.4% amplification in antineoplastic cytotoxicity, evidenced by the suppression of cellular viability from 116% down to 61% in the MTT analysis. This inverse correlation underscores a pivotal pharmacological principle, the robust structural entrapment provided by the chitosan matrix which initially constrains immediate curcumin diffusion, serves as the primary mechanism for mitigating premature drug degradation. Consequently, this sustained-release profile facilitates a prolonged, more efficient intracellular exposure, ultimately yielding heightened and sustained therapeutic efficacy against malignant cell lines [52].

Immunomodulatory

Fong *et al*. demonstrated that a specific number of chitosans (3-10 kDa, 98% DDA; 10 and 190 kDa, 80% DDA, block-acetylated) in particulate form imitate this model of macrophage infection by intracellular pathogens. Chitosans with a minimum of 3000 Da consecutive GlcN residues (about 18 tandem GlcNs) produced a strong type 1 IFN response in macrophages at modest doses without activating the inflammasome. At larger concentrations, the identical chitosans triggered the inflammasome but did not elicit a type 1 IFN response [53]. These intriguing findings imply that macrophages use similar sensing systems to create anti-inflammatory signals following chitosan particle or intracellular pathogen uptake. Lysosomal escape was identified as the unique and common mimetic event. While it has been documented that chitosan intrinsic characteristics play an important role in the release of pro- or anti-inflammatory signals, the effect of dosage has only recently been identified. This newly discovered dose impact shows the possibility of fine-tuning chitosan use, not only in terms of structural features, but also in dose, to elicit or prevent specific immunomodulatory responses for a desired biomedical purpose [54].

CONCLUSION

The complete assessment of chitosan modification strategies including chemical and physical, emphasizes the biopolymer's versatility as a top contender for improved drug delivery. Chemical modifications, including cross-linking (both synthetic and natural), carboxylation, acylation and quaternization have shown promise in overcoming chitosan's intrinsic limitations, such as limited solubility at neutral pH and rapid enzymatic breakdown. The combination of Schiff base chemistry and graft copolymerization has allowed for the generation of "smart" stimuli-responsive carriers that provide accurate, site-specific release, especially in acidic tumor microenvironments. Simultaneously, physical alterations like as gamma irradiation and ultrasonic treatment offer a "green" alternative for creating low-molecular-weight chitosan (LMWC) with increased bioactivity and purity, which is required for high-quality pharmaceutical applications. The biological properties discussed are antibacterial, antioxidant, anticancer and immunomodulatory show that modified chitosan is more than just an inert carrier, it is an active medicinal ingredient. Chitosan derivatives significantly improve the therapeutic index while maintaining excellent biocompatibility and low systemic toxicity from increasing the bioavailability of hydrophobic drugs such as curcumin and paclitaxel to acting as a potent vehicle for gene delivery via electrostatic complexation. Despite tremendous progress shown in recent literature, various issues remain to be solved for the successful clinical translation of chitosan-based delivery systems. There is a critical need for the standardization of modification protocols to ensure batch-to-batch reproducibility in terms of the degree of deacetylation (DD) and molecular weight distribution which are pivotal for predictable drug release kinetics. While synthetic treatments such as glutaraldehyde and ECH are effective, their residual toxicity is still a worry.

While Schiff base and pH-responsive systems seem promising, the development of multi-stimuli-responsive nanocarriers (which include pH, temperature and ultrasonic sensitivity) could improve the precision of "on-demand" drug release. As chitosan derivatives become more complicated (hybrid-grafted or radiation-modified), navigating the regulatory landscape for FDA/EMA approval will require more thorough clinical data on their immunomodulatory effects at varied doses. There is a huge difference

between successful in vitro outcomes and in vivo performance. Future research should use complicated animal models to further understand the metabolic pathways and long-term removal of modified chitosan nanoparticles from the systemic circulation. To ensure optimum patient safety, future research should focus on natural cross-linkers (e.g., Genipin, Vanillin, Tannic Acid) and radiation-based processing. As a summary, the transformation of chitosan from a simple biopolymer to a sophisticated, multi-functional biomaterial ushers in a new era in nanomedicine. By bridging the gap between creative chemical engineering and biological efficacy, modified chitosan has the potential to revolutionize targeted therapy for chronic diseases and infectious pathogens.

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