REVIEW

A COMPREHENSIVE REVIEW ON β-CHITOSAN FROM CEPHALOPOD WASTES: PROCESSING, PHYSICOCHEMICAL PROPERTIES, AND ITS BIOMEDICAL POTENTIAL

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Abstract

Chitosan is derived from chitin through deacetylation. Extensive research has focused on extracting chitin from affordable sources. Commercially available chitosan products are primarily derived from α-chitin, extracted from crustacean exoskeletons, which are rich in inorganic material. In contrast, β-chitosan is derived from β-chitin sourced from cephalopods such as squid pens with negligible mineral content. This natural purity of β-chitin sources eliminates the need for aggressive chemical demineralisation, making the production process more environmentally friendly. Despite promising attributes, β-chitosan has received limited attention in the scientific literature compared to α-chitosan. A comprehensive literature search was conducted to address this knowledge gap by providing an extensive review to elucidate the structural and functional distinctions of β-chitosan. The article evaluates its biomedical efficacy, highlighting antibacterial activity against multidrug-resistant pathogens in preclinical models. The antioxidant, antitumor, and anticoagulant properties are also discussed. The findings demonstrate the potential of β-chitosan as a versatile, high-performance, and sustainable biopolymer for advanced biomedical applications.

Keywords: *biopolymer; chitin; β-chitosan; seafood waste*

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1. Introduction

Recent breakthroughs in biomedical sciences have created a surge in demand for novel biocompatible materials, with biopolymers emerging as prime candidates due to their exceptional properties, such as hydrophilicity, biocompatibility, biodegradability, abundance, and the presence of derivatizable functional groups [1]. Marine biopolymers extracted from seafood discards, previously regarded as waste, are being repurposed as renewable biomaterials. This approach offers a sustainable solution to mitigate waste generation and environmental degradation [2]. Chitin is the second most abundant polysaccharide globally, following closely behind cellulose. Living organisms produce 10^{10}–10^{12} tonnes of chitin [3]. Chitosan is obtained by the deacetylation of chitin and has become a cynosure across the biomedical field due to its unique physical, chemical, and biological properties conferred by its functional amino (NH_2) and hydroxyl (OH) groups. The escalating interest in chitosan is evident from the exponential growth in publications and patents over the years, as illustrated in Figure 1 [4].

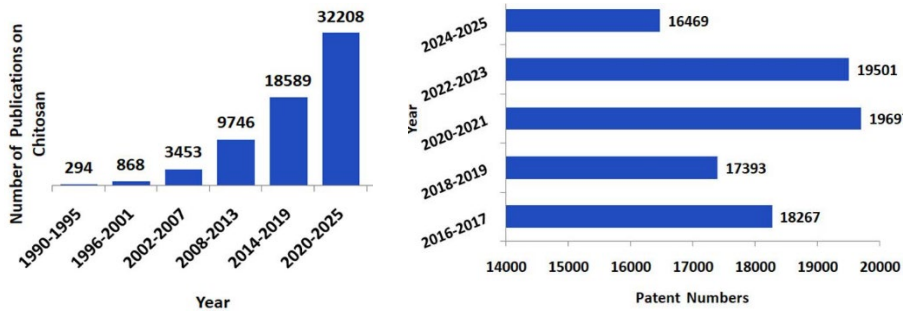

Figure 1. The number of: (a) publications from 1990 to 2025 retrieved from the PubMed database using the keyword 'chitosan' in the title; (b) patents granted on chitosan from 2016 to 2025 (data obtained from the WIPO PATENTSCOPE database) using the keyword 'chitosan'.

This trend emphasises the immense potential of chitosan for applied research and industrial innovation. Cephalopod-derived β-chitin, with its parallel-aligned *N*-acetyl glucosamine (GlcNAc) chains, displays distinct properties, including enhanced reactivity, improved solvent interactions, and significant bactericidal activity, making it more desirable than α-chitin derived from crustaceans for various applications [5]. The unique functionality of β-chitosan makes it an attractive candidate for biomedical applications, yet it remains a relatively underexplored area of research, with limited studies and reports available. This disparity in research focus is evident in Figure 2, which shows the significantly lower volume of scientific output on β-chitosan compared to the extensive literature on the α-polymorph. Despite its promising attributes, β-chitosan has received only a fraction of the academic attention devoted to its crustacean-derived counterpart. While general reviews on chitosan often treat the β-chitosan polymorph as a minor footnote compared to the dominant α-chitosan form, this review provides a distinct perspective by positioning β-chitosan as a specialised high-performance biopolymer. The novelty of this work lies in its critical synthesis of the structure-property-function triad specific to the β-chitosan configuration. Unlike generic summaries, this review correlates the unique parallel-chain supramolecular architecture of β-chitosan with its superior reactivity and biomedical efficacy.

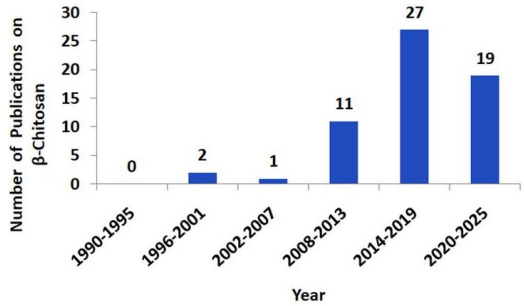

Figure 2. Number of publications from 1990 to 2025 retrieved from the PubMed database using the keyword 'β-chitosan' in the title.

Furthermore, it addresses the practical challenges of sourcing and processing cephalopod waste, offering a realistic assessment of the scalability and commercial viability of β-chitosan that is often absent from purely laboratory-focused studies. The primary reason is the limited availability of sources for extraction. In the present scenario, the valorisation of marine resources has increased the raw material used for β-chitosan production. It is imperative that the waste from seafood processing industries, namely cephalopod endoskeletons, be used as an economically and environmentally valuable resource for producing β-chitosan. Despite the growing interest in β-chitosan as a sustainable biomaterial, there is a notable lack of comprehensive reviews that synthesise the latest research in this area. This knowledge gap motivated the development of this review article. This review provides a comprehensive analysis of studies published between 2000 and 2025, highlights the latest advancements in the processing and application of β-chitosan, and offers a state-of-the-art overview of β-chitosan, including its structure, sources, preparation methods, properties, and biomedical applications.

2. Molecular Structure of β-Chitosan

From a structural perspective, chitin is a linear, long-chain homopolymer composed exclusively of β-(1→4)-linked units of *N*-acetyl-D-glucosamine. It is structurally identical to cellulose, except that the hydroxyl group at the C-2 position of each glucose unit is replaced by an acetamido group [6] (Figure 3).

Depending on its source, chitin exists in three different polymorphic forms: α, β, and γ-chitin. These forms differ in the orientation and association of individual chains within fibres. Each form is characterised by a unique arrangement of macromolecular chains: specifically, antiparallel in α-chitin, wherein the fibrils are tightly packed and bonded; parallel-orientated sheets in β-chitin; and γ-chitin, which is a crystalline, structural mixture of the α and β-forms [7, 8] (Figure 4).

The transition from β-chitin to β-chitosan is governed by the supramolecular architecture of the parent polysaccharide. Since β-chitosan is derived from the deacetylation of β-chitin, it retains the characteristic parallel chain alignment of its precursor. Moreover, β-chitosan exhibits a unique structure characterised by the parallel arrangement of polymer chains and reduced intermolecular packing, which increases the accessibility of hydrophilic groups, thereby facilitating increased hydrogen bonding with water. An appropriate hydration level, combined with reduced intermolecular attachment and increased chain mobility, renders the β-chitosan structure more reactive [9].

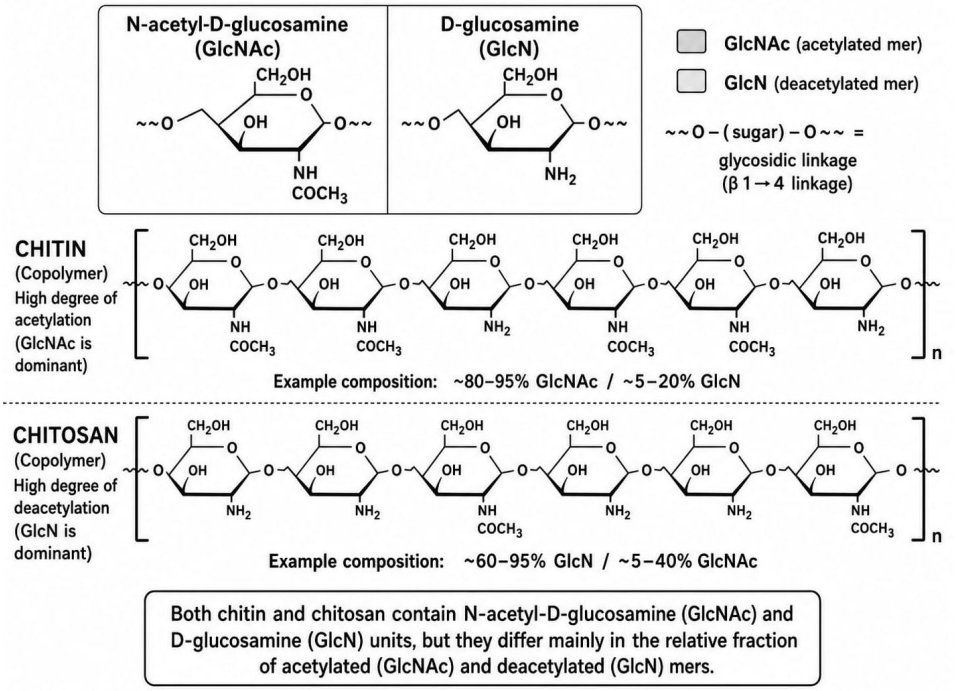

Figure 3. Structure of chitin and chitosan. This image was created with BioRender (https://biorender.com/).

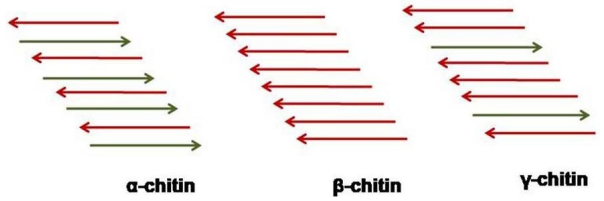

Figure 4. Schematic representation of the three polymorphic forms of chitin, which express differences in chain orientation.

The structural transition from β-chitin to β-chitosan is directed by the unique supramolecular architecture of the parent polysaccharide. In contrast to α-chitin, which consists of tightly packed antiparallel chains stabilised by strong intermolecular hydrogen bonds, β-chitin is characterised by a parallel arrangement of chains. This parallel orientation results in looser crystalline packing and weaker inter-sheet interactions, facilitating reagent penetration during deacetylation. Due to this topochemical relationship, β-chitosan retains the parallel chain orientation of the original β-chitin when deacetylation is performed under heterogeneous conditions. Moreover, this also differentiates it significantly from α-chitosan, which commonly crystallises in the thermodynamically stable antiparallel arrangement. Consequently, β-chitosan derived from β-chitin generally exhibits lower crystallinity, higher porosity, and enhanced solubility and reactivity compared to its α-counterpart. However, this loose crystalline packing, while beneficial for chemical reactivity, reduces mechanical rigidity and thermal stability relative to the dense α-form, potentially limiting its use in load-bearing applications without further modification [10].

3. Occurrence of β-Chitin

Chitin, a key component of invertebrate skeletons, is found in various forms across arthropods, molluscs, and annelids [11]. β-chitin serves as the exclusive natural precursor for the production of β-chitosan. Therefore, a detailed understanding of β-chitin's structural configuration is critical, as the supramolecular architecture of the source material is retained during the deacetylation process and dictates the unique physicochemical properties observed in the final β-chitosan polymer. β-Chitin is predominantly sourced from molluscs, including squid pens, shells of clams and oysters, and cuttlefish bones, as shown in Figure 5. The occurrence of β-chitin is restricted to specific taxonomic groups, making the selection of raw material a critical factor for β-chitosan production. The most commercially viable sources of β-chitin are cephalopods, particularly squid pens and cuttlefish bones. The gladius (pen) of squid, such as *Loligo duvauceli* and *Sepioteuthis lessoniana*, consists largely of β-chitin.

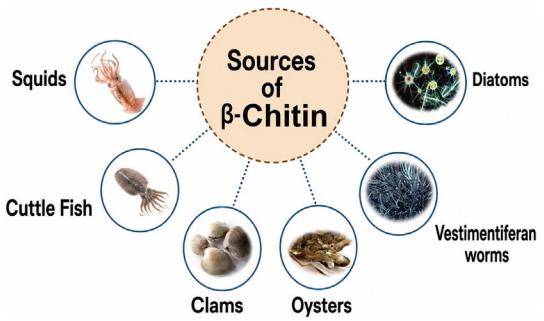

Figure 5. Overview of the occurrence of β-chitin in living organisms. This image was created with BioRender (https://biorender.com/).

Quantitative analyses have reported that the chitin content in squid pens ranges from approximately 15 to 30% of dry weight, varying by species and season [12]. Similarly, the cuttlebone of cuttlefish is a rich source, with β-chitin content reported to range from 10 to 30% [13]. Beyond cephalopods, β-chitin has been identified in the marine tubes produced by vestimentiferan worms, pogonophorans, and aphrodite chaetae [14]. The diatom *Thalassiosira fluviatilis* has also been reported to contain pure β-chitin in its monocrystalline spine. Reports of β-chitin in bivalves, such as clams and oysters, refer to the organic periostracum layer rather than the calcified shell itself; however, quantitative data on the β-fraction in these organisms are often limited [11].

Unlike crustacean shells (rich in α-chitin and calcium carbonate), squid pens offer a naturally high yield of the β-polymorph with negligible mineral content [12]. β-Chitosan is intrinsically linked to the availability of these raw materials. While the global crustacean sector provides abundant α-chitin waste, the global squid and cuttlefish fisheries provide a distinct, massive, and underutilised β-chitin feedstock. Annual global squid catches exceed 4 million tonnes, with the internal pen (gladius) constituting approximately 2–4% of the total body weight [11, 12]. This translates to a potential supply of over 100 000 tonnes of squid pen waste annually. This substantial volume of renewable biomass, often discarded as processing waste, underscores the high practical potential of cephalopod sources for sustainable, industrial-scale β-chitosan production, offering a 'waste-to-wealth' perspective distinct from the crustacean supply chain. Nevertheless, a critical logistical challenge persists. Unlike crustacean waste, which processing plants typically concentrate, squid pens are often discarded at sea or during initial sorting aboard vessels.

This lack of established collection infrastructure poses a significant barrier to obtaining the consistent, high-volume feedstock required for commercial viability. Despite the substantial volume of potential waste, the industrial availability of β-chitin remains constrained compared to the ubiquity of crustacean waste. This limitation is due to specific geographic and industrial factors: (i) squid and cuttlefish fisheries are predominantly found in specific regions (particularly the Southwest Atlantic, Northwest Pacific, and Indian Ocean), leading to an uneven global distribution of raw materials; (ii) these fisheries exhibit strong seasonal variability, resulting in inconsistent year-round supply; and (iii) unlike crustacean processing, which has established waste collection streams, squid pens are often discarded at sea or lost during processing due to inadequate collection infrastructure. Consequently, while the theoretical waste stream is large, the accessible volume for consistent β-chitosan manufacturing is currently lower than that for α-chitosan, creating a bottleneck for commercial scalability [15].

4. Biosynthesis of β-Chitin

The biosynthesis of chitin relies on a highly conserved enzymatic pathway shared across diverse organisms, including both arthropods (crustaceans) and cephalopods. Although the final structural polymorphs (α vs. β) differ due to post-synthetic assembly in the extracellular matrix, the biochemical steps involved in polymer formation are identical. This universal mechanism initiates with glucose and storage carbohydrates, such as trehalose and glycogen, as the primary substrates for chitin production.

The biosynthesis of β-chitin in cephalopods follows a complex, multi-step enzymatic pathway that is conserved across invertebrates. Like arthropods, cephalopods initiate biosynthesis with glucose and storage carbohydrates, utilising a shared metabolic sequence to produce the fundamental chitin polymer. This universal pathway is outlined below [14] (Figure 6).

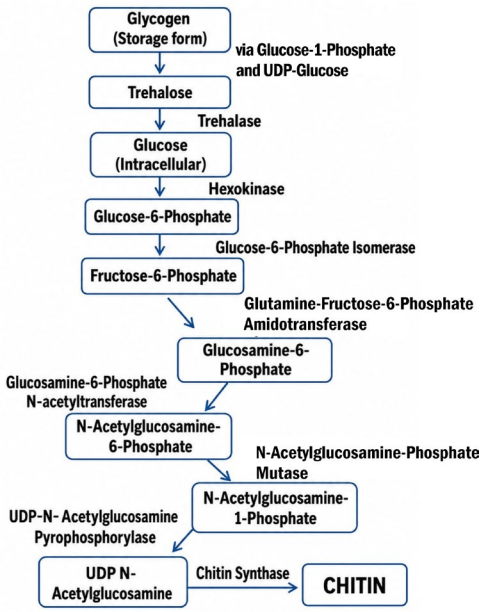

Figure 6. Step-by-step schematic representation of the chitin biosynthetic pathway.

i. Glycogen is converted to glucose-1-phosphate by glycogen phosphorylase, which can then participate in trehalose biosynthesis via the UDP-glucose pathway.
ii. Trehalase, an enzyme widespread in insect tissues, breaks down trehalose into two intracellular glucose molecules.
iii. Glucose is converted to glucose-6-phosphate, which is then isomerised to fructose-6-phosphate by glucose-6-phosphate isomerase.
iv. The chitin biosynthetic pathway diverges from this point, with glutamine fructose-6-phosphate aminotransferase catalysing the first committed step in amino sugar biosynthesis.
v. Fructose-6-phosphate is converted to *N*-acetylglucosamine (GlcNAc) phosphate through a series of amination, acetyl transfer, and isomerisation reactions, catalysed by several enzymes, including phospho-*N*-acetylglucosamine mutase.
vi. This compound is then transformed into UDP-*N*-acetylglucosamine, serving as the substrate for chitin synthesis.
vii. Chitin synthase ultimately converts UDP-*N*-acetylglucosamine into chitin.

The biochemical enzymatic pathway for the synthesis of UDP-*N*-acetylglucosamine and its polymerisation into chitin chains is conserved and identical across organisms that produce both α- and β-chitin. The divergence between the two polymorphs occurs after synthesis, during the supramolecular assembly and crystallisation phase. The formation of specific polymorphs, α and β-chitin, is determined by the supramolecular assembly and crystallisation control that occurs in vivo [5, 8]. The divergence arises from the distinct biosynthetic context of the organism. It should be noted that in crustaceans, chitin chains are extruded into a highly regulated, mineral-rich extracellular matrix where specific chitin-associated proteins and the physical constraints of the exoskeleton drive the polymer chains to align in an antiparallel arrangement, forming the dense, rigid α-chitin. In contrast, in cephalopods (e.g., squid and cuttlefish), the biosynthesis occurs within a softer, organic matrix (the pen or cuttlebone) with minimal mineralisation. In this environment, the absence of rigid calcification pressure and the specific interaction with the organic matrix allow nascent chitin chains to align in parallel upon crystallisation. This organism-specific control over chain orientation during deposition yields the β-polymorph, characterised by its looser packing and higher reactivity. Therefore, it is important to clarify that although the enzymatic biosynthetic pathway is identical in α- and β-chitin-producing organisms, the structural polymorphism is determined solely by differences in the extracellular matrix environment during crystallisation [16].

5. Preparation Route of β-Chitosan from β-Chitin

The extraction of β-chitosan from marine by-products is a pivotal process that determines the final polymer's quality, yield, and applicability. A critical factor influencing the extraction route is the unique microstructure of the precursor, β-chitin. Unlike the tightly packed antiparallel chains of α-chitin, the parallel arrangement and weaker hydrogen bonds in β-chitin render the polymer matrix significantly more accessible to chemical reagents. This structural characteristic directly facilitates extraction, resulting in superior reaction kinetics and higher yields than the α-form. Specifically, regarding deacetylation kinetics, β-chitin exhibits superior reactivity. Under typical heterogeneous alkaline conditions (e.g., concentrated NaOH at elevated temperatures), the conversion of β-chitin to β-chitosan achieves a degree of deacetylation (DDA) of 70% within 1 hour, whereas α-chitin requires approximately 6 hours to achieve a similar DDA under comparable conditions [17]. Furthermore, the overall processing protocol for β-chitosan

is less demanding; utilising squid pens requires lower reagent concentrations (1 N NaOH) and shorter reaction times (24 h) compared to the production of α-chitosan from crustacean shells, which necessitates a two-step process involving demineralisation with 2 N HCl followed by deacetylation with 1 N NaOH for 48 h [18]. Building on these structural advantages, research by Roshan Ara Begum *et al.* [12] demonstrated that squid pens, rich in β-chitin, allow for a simplified preparation protocol (Figure 7).

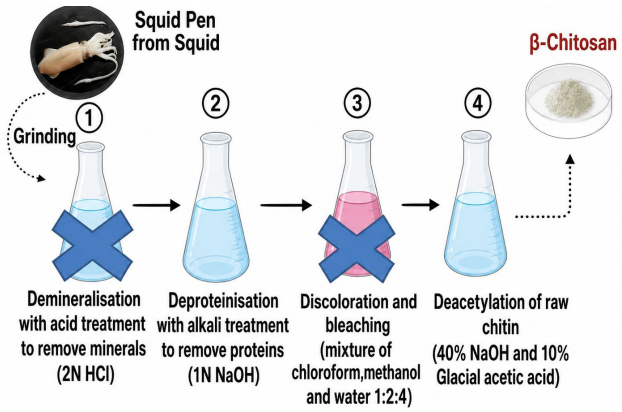

Figure 7. Schematic representation of the preparation route for β-chitosan from squid pen. The 'X' symbol represents the omission of that particular step. This image was created with BioRender(https://biorender.com/).

Due to the inherently low mineral content of the source, the demineralisation step can be omitted, leaving deproteinisation and deacetylation as the only necessary steps to isolate chitosan. This modification not only streamlines the process but also preserves the polymer's native properties. Moreover, β-chitin's loosely packed crystallographic structure makes it more amenable to alkali deproteinisation than α-chitin. Chaussard and Domard [19] reported a marked difference in protein removal kinetics, where squid pens exhibited rapid protein elimination, with 90% removal achieved within 15 minutes, whereas shrimp shells required over 2 hours to achieve a comparable extent of deproteinisation. Therefore, squid pen deproteinization is a cost-efficient process, characterised by reduced reagent consumption and shorter reaction times, making it a highly viable option for industrial-scale processing. However, this high reactivity is a double-edged sword and necessitates stringent control of reaction conditions to circumvent excessive depolymerisation and polydispersity. Moreover, without a calcified shell, squid pens are highly susceptible to enzymatic autolysis and microbial spoilage. Rapid processing or immediate preservation is therefore required to prevent polymer degradation prior to extraction [20].

6. Physicochemical Properties of β-Chitosan

The physicochemical properties of β-chitosan are highly interrelated and play a crucial role in determining its functionality, applicability, and interactions with other molecules. The key intrinsic polymer parameters that drive these performance-related properties are the degree of deacetylation (DDA) and molecular weight (MW). Solubility is not an independent primary parameter but rather a resultant property that depends on these underlying intrinsic characteristics and the solution environment. Understanding these interrelationships is essential for tailoring β-chitosan for specific biomedical applications.

6.1. Degree of Deacetylation

The degree of deacetylation (DDA) is a critical chemical characteristic that significantly impacts the performance and functionality of chitosan in various applications. DDA influences the solubility, charge density, and biological interactions of chitosan, thereby determining its suitability for specific uses [21, 22]. Chitosan is commercially produced by deacetylation of chitin, a process that removes acetyl groups from the chitin polymer chain. This process determines the content of free amino groups in the polysaccharide. Chitosan offers two significant advantages over chitin: (i) solubility - chitosan readily dissolves in dilute acetic acid, whereas chitin requires highly toxic solvents; (ii) reactivity - chitosan possesses free amine groups, which serve as active sites in various chemical reactions. Notably, β-chitosan exhibits an enhanced DDA compared to α-chitosan [12]. This is attributed to the fact that β-chitin is much more amenable to *N*-deacetylation than the α-form. Furthermore, research has shown that β-chitin's crystallographic structure is less rigid and characterised by a lower density of intermolecular hydrogen bonds than that of α-chitin. This reduced bonding network facilitates the penetration of alkali solutions [23–25]. Rhazi *et al.* [26] found that β-chitin exhibits greater reactivity to alkali treatments compared to α-chitin. Consequently, β-chitosan achieves a higher DDA within a shorter treatment time of 2 hours. Accurate determination of DDA is achieved using spectroscopic techniques such as Nuclear Magnetic Resonance (NMR), Fourier Transform Infrared Spectroscopy (FT-IR), and ultraviolet-visible (UV-Vis) spectroscopy. Among these, NMR is often preferred for its precision in defining the acetylation pattern, which is critical for establishing reliable structure-property relationships and comparing the reactivity of β-chitosan to other polymorphs [27].

6.2. Molecular Weight

Molecular weight (MW) significantly influences the physicochemical properties and biological functions of chitosan. Based on molecular weight ranges, chitosan is categorised into three types: high molecular weight chitosan (HMWC, > 700 kDa), medium molecular weight chitosan (MMWC, 150–700 kDa), and low molecular weight chitosan (LMWC, < 150 kDa) [28]. β-chitosan is obtained from cephalopod sources, such as squid pens and cuttlefish bones, and falls within the 500–700 kDa range, placing it in the MMWC category [29, 30]. This distinguishes it from many crustacean-derived α-chitosans, which often retain higher molecular weights (> 1000 kDa) unless intentionally depolymerised. The classification of β-chitosan as ‘medium’ molecular weight is relative to the native starting material. While the precursor β-chitin is a high molecular weight biopolymer, the conversion process results in a reduction in chain length, which is driven by the structural susceptibility of β-chitin; its loose parallel chain arrangement offers less steric hindrance compared to the tightly packed antiparallel structure of α-chitin. Apparently, during deacetylation, the polymer chains are more exposed to the alkaline environment (conc. NaOH), thereby promoting alkaline hydrolysis and oxidative chain scission [17, 31]. Although β-chitosan production avoids the harsh acidic demineralisation step required for α-chitosan, thereby avoiding acid-induced degradation, the inherent reactivity of the β-polymorph makes it specifically susceptible to alkaline degradation. This combination of structural accessibility and processing conditions yields the observed medium-molecular-weight β-chitosan [17, 28].

6.3. Solubility

Chitosan solubility is chiefly governed by the protonation of amino groups in acidic media (pH < pKa ~6.5). Distinct from the α-form, β-chitosan exhibits significantly enhanced

solubility in dilute organic acids. Studies indicate that β-chitosan dissolves rapidly and completely in 1% (v/v) acetic acid at room temperature, often forming solutions with lower viscosity than α-chitosan of similar molecular weight [23]. This superior performance is attributed to the weaker intermolecular interactions and lower crystallinity of the β-chitosan polymorph, which facilitates solvent penetration even at moderate DDA. For instance, Tolaimate *et al.* [24] observed that β-chitosan exhibits higher solubility in aqueous acetic acid than its α-counterpart, a property largely independent of the specific deacetylation conditions used. Furthermore, the high DDA achieved in β-chitosan (exceeding 85%) promotes solubility in weak acids such as lactic acid and makes it highly suitable for biomedical processing applications [28]. A comprehensive comparative analysis of the physicochemical characteristics and processing routes of β-chitosan versus α-chitosan is summarised in Table 1.

7. Functional Properties of β-Chitosan

Chitosan is an amino-containing polysaccharide that exhibits distinct physicochemical characteristics that confer unique functional properties. Its diverse biological attributes, including biocompatibility, biodegradability, and non-toxicity, render it an ideal biomaterial for in vivo applications. Its solubility, MW, and DDA are crucial factors influencing these properties [32].

7.1. Water Binding Capacity

The water binding capacity (WBC) of chitosan is a crucial functional property that is intrinsically linked to its molecular structure and intermolecular interactions. In β-chitosan, the parallel arrangement of polymer chains and the resulting weaker intermolecular hydrogen bonds lead to lower crystallinity and looser chain packing compared to the dense, antiparallel structure of α-chitosan. Besides, this open, porous microstructure facilitates the physical penetration and retention of water molecules within the polymer matrix. Furthermore, the higher DDA observed in β-chitosan increases the availability of hydrophilic functional groups, such as amino and hydroxyl groups, for hydrogen bonding with water. These structural advantages translate to superior hydration performance. Quantitatively, Subhapradha *et al.* [33]reported a WBC of 608% for β-chitosan extracted from the squid pen *Sepioteuthis lessoniana* [33]. This value is significantly higher than the WBC generally reported for α-chitosan, which is often constrained by its high crystallinity and tighter chain association. Studies also indicate that the increased DDA and decreased crystallinity of β-chitosan are the primary drivers of this enhanced water uptake, making it particularly effective for applications requiring high swelling or moisture retention [34, 35].

7.2. Fat Binding Capacity

The fat binding capacity (FBC) of chitosan is governed by an interfacial mechanism occurring in aqueous emulsions, rather than by direct binding to neutral triglycerides. Under the acidic conditions required for solubilisation, chitosan's amino groups are protonated to form cationic $-NH_3^+$ ions. These cationic sites interact electrostatically with anionic components present at the oil-water interface, specifically free fatty acids (FFAs) and phospholipids. Consequently, the structural properties of β-chitosan, its high DDA, and lower crystallinity make it particularly effective for this mechanism. The high DDA provides a greater density of positive charge for electrostatic interaction, while the loose

Table 1. Comparative overview of the physicochemical characteristics and processing of β-chitosan and α-chitosan.

Feature	β-chitosan	α-chitosan	Remarks / implications	[Ref]
primary source	squid pens (endoskeleton)	shrimp/crab shells (exoskeleton)	β-sources have negligible mineral content	[3, 11–13]
chain arrangement	parallel alignment	antiparallel alignment	parallel packing results in weaker intermolecular forces	[5, 7–10]
crystallinity	lower crystallinity, loose packing	higher crystallinity, tight packing	lower crystallinity increases reactivity and solubility but reduces mechanical strength	[5, 9, 10]
degree of deacetylation	higher (achieved rapidly)	moderate (requires longer duration)	high DDA enhances positive charge density, improving biological interactions and solubility	[12, 17, 23, 24, 26]
solubility	high (rapid dissolution in dilute acids)	moderate (often requires concentrated acids)	β-chitosan forms low viscosity solutions suitable for biomedical processing	[23, 24, 28]
molecular weight	medium (500–700 kDa)	high (> 700 kDa, often > 1000 kDa)	β-chitosan is more susceptible to alkaline degradation during processing due to structural accessibility	[17, 28, 30, 31]
water binding capacity (WBC)	high	low	high WBC is driven by high DDA and porosity, beneficial for wound healing applications	[32–34]
fat binding capacity (FBC)	high	low	high FBC is attributed to high DDA and a porous structure facilitating electrostatic interactions	[33, 34]
processing route	deproteinisation → deacetylation	demineralisation → deproteinisation → deacetylation	β-chitosan production is more environmentally friendly and cost-effective	[12, 17–20]
reaction kinetics	fast (deacetylation: ~1–2 hrs; deproteinisation: ~15 mins for 90%)	slower (deacetylation: ~4–6 hrs; deproteinisation: > 2 hrs)	reduced energy and reagent consumption for β-form	[17, 19, 20]
supply chain	seasonal, geographically limited, less collection infrastructure	abundant, established global supply chain	scalability is the major bottleneck for β-chitosan, despite its lower processing costs	[3, 15]

parallel packing enhances porosity and accessibility to the oil-water interface. As a result, β-chitosan demonstrates a superior FBC of 3.9 $g_{oil}/g_{chitosan}$, significantly higher than the 2.7 $g_{oil}/g_{chitosan}$ observed for α-chitosan [36].

7.3. Antibacterial Activity

The antibacterial activity of chitosan is mediated by multiple mechanisms that are significantly influenced by its MW. While electrostatic attraction between protonated amine groups and negatively charged components of bacterial membranes is a primary mechanism [37], MMW chitosan, such as β-chitosan (500–700 kDa), exerts effects through the formation of a polymeric film on the cell surface. This surface coating acts as a physical barrier, impeding the transport of essential nutrients and the excretion of metabolic waste, ultimately leading to cell death [38]. Research has demonstrated that β-Ch-ZnO-NPs exhibit broad-spectrum antimicrobial activity, inhibiting the growth of *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Candida albicans* [39]. An assessment of the antibacterial efficacy of β-chitosan against various pathogens, including *Salmonella enterica*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Bacillus cereus*, revealed its potent inhibitory effects. At a concentration of 49 mg/mL, β-chitosan exhibited significant antibacterial activity, with zones of inhibition ranging from 0.5 to 2.3 mm against the tested pathogens [35]. The antibacterial efficacy of LMW water-soluble chitosan β-chitosan against multidrug-resistant bacterial strains, including *S. aureus* and *Pseudomonas aeruginosa*, was assessed. The results showed that β-chitosan exhibited promising antibacterial activity compared to α-chitosan, effectively inhibiting bacterial growth in both *in vitro* and *in vivo* studies. The structural flexibility of β-chitosan, which allows for increased contact with bacterial surfaces in aqueous solution, coupled with its ease of protonation, contributes to its enhanced antibacterial efficacy in these experimental settings [40].

Besides, studies have demonstrated the antibacterial efficacy of phosphorylated chitosan derived from *Sepia kobiensis* against various pathogens. Particularly, this modified chitosan exhibited significant inhibition zones of 17, 16, and 7 mm against *Proteus vulgaris*, *Staphylococcus aureus*, and *Vibrio cholerae*, respectively, when tested at the 100% stock concentration (5 mg/mL), wherein lower percentage values represent serial dilutions of this stock [40, 41]. Nevertheless, the precursor β-chitin extracted from *Phyllobates bicolor* shells has been reported to display intrinsic antibacterial activity against both Gram-positive and Gram-negative pathogens at a relatively low concentration of 200 μg/mL [42]. This highlights the potential of this marine source for yielding bioactive β-chitosan derivatives. As illustrated in Figure 8, the antimicrobial mechanism of β-chitosan is a multi-stage process. Initially, the positively charged chitosan interacts with the negatively charged bacterial cell surface. In higher-molecular-weight β-chitosan, this interaction facilitates the formation of a polymeric film or barrier that impedes nutrient transport. Subsequently, the polymer disrupts the cell wall integrity, causing lysis and the leakage of intracellular components. Finally, chitosan penetrates cells, where it binds to DNA and inhibits transcription and RNA synthesis, thereby preventing protein synthesis and leading to cell death.

In comparative in vitro studies, β-chitosan often exhibits superior antibacterial efficacy against representative strains compared to α-chitosan. For instance, studies have shown that β-chitosan provides more effective inhibition against both Gram-negative (e.g., *Escherichia coli*) and Gram-positive (e.g., *Staphylococcus aureus*) bacteria than α-chitosan of comparable molecular weight [18]. This enhanced performance is due to the

distinct supramolecular architecture of β-chitosan. Unlike α-chitosan, which is characterised by tightly packed antiparallel chains and high crystallinity, β-chitosan possesses a looser crystalline structure derived from the parallel chain alignment of β-chitin. This reduced crystallinity and conformational rigidity significantly increase the accessibility of the polymer's functional groups [18]. Furthermore, β-chitosan achieves a higher DDA than α-chitosan, leading to a greater density of protonatable amine groups and improved solubility. As a result, β-chitosan exhibits a higher positive charge density in solution, enabling stronger electrostatic binding to bacterial surfaces. Its inherent structural flexibility also enables it to cover a larger surface area of the cell membrane, enhancing membrane disruption and permeability compared to the more rigid α-form of chitosan. While the *in vitro* antibacterial efficacy of β-chitosan is well documented, it is important to note that a significant portion of this data relies on lab-scale extractions from various cephalopods. The variability in DDA and molecular weight across different extraction protocols leads to inconsistent antibacterial performance. Furthermore, most studies are conducted in controlled media, whereas the ionic environment of real physiological systems may alter the polymer's charge density and, apparently, its efficacy [14, 37]. Therefore, future research must standardise these extraction parameters to ensure reproducibility before clinical translation.

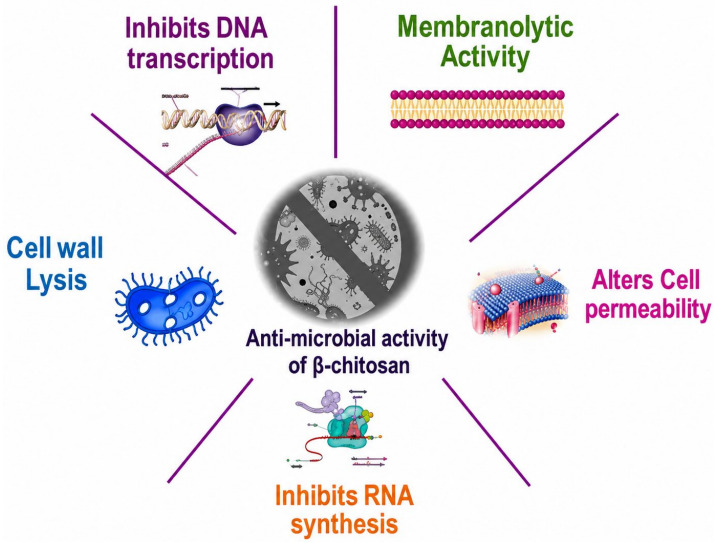

Figure 8. Mechanism of the antimicrobial properties of β-chitosan. This image was created with BioRender (https://biorender.com/).

7.4. Antioxidant Activity

The antioxidant properties of chitosan are well-documented. Preclinical studies with β-chitosan derived from Sepia aculeata cuttlebones demonstrate potential activity by neutralising DPPH and hydroxyl radicals at rates of 88.6 and 72%, respectively [13]. While chitosan and its derivatives possess antioxidant properties attributed to the scavenging of oxygen radicals (e.g., hydroxyl, superoxide, and alkyl radicals) and stable DPPH radicals via hydrogen donation or electron transfer [43], the specific efficacy of β-chitosan is closely linked to its structural organisation. With regard to molecular weight, native β-chitosan has a medium molecular weight (500–700 kDa), and studies on antioxidant activity frequently utilise hydrolysed or depolymerised chitosan fractions.

Research indicates that LMW β-chitosan with an MW of 20–30 kDa exhibits enhanced activity compared to HMW [44]. This enhancement is attributed to the increased solubility of smaller chains and a higher density of exposed reducing ends available for radical scavenging. In addition, although α- and β-chitosan contain the same functional group types (amino and hydroxyl), β-chitosan often displays superior antioxidant capacity due to differences in physical accessibility. The looser crystalline packing and parallel chain orientation of β-chitosan facilitate greater access of free radicals to these active sites compared to the tightly packed antiparallel structure of α-chitosan. Additionally, the higher DDA of β-chitosan contributes to its improved solubility under aqueous assay conditions, enabling more efficient electron transfer and interaction with reactive oxygen species.

7.5. Antitumour Activity

Chitosan, a cationic polymer with inherent mucoadhesive properties, acts as both a therapeutic agent and a vehicle for delivering anticancer drugs directly to tumour cells [45]. β-chitosan exhibits antiproliferative activities through a multi-modal mechanism of action. These include: (i) antiangiogenic effects, which starve tumours of nutrients; (ii) immunoenhancement, such as the activation of T-cells and natural killer cells; (iii) antioxidant defence; (iv) the induction of apoptosis; and (v) the regulation of enzymatic activity [46]. This combination of direct cytotoxicity and immune system activation underscores the potential of β-chitosan in cancer therapy. Subhapradha and Shanmugam [47] investigated the fabrication of β-chitosan nanoparticles (β-CNP) and their anticancer potential against human hepatoma (HepG2) cells. The study assessed various biochemical parameters, including TBARS (thiobarbituric acid reactive substances), lipid hydroperoxides, and enzymatic and non-enzymatic antioxidants. The results demonstrated the anticancer properties of β-CNP in HepG2 cells, suggesting that β-CNP is a promising candidate for further investigation in the management of hepatocellular carcinoma. The hepatoprotective effects of β-chitosan derived from the gladius of *Sepioteuthis lessoniana* were investigated against carbon tetrachloride-induced oxidative stress in Wistar rats. The results showed that β-chitosan exhibited significant antihepatotoxic activity by markedly enhancing hepatic and circulatory antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), as well as reducing glutathione (GSH) levels. β-chitosan decreased malondialdehyde levels, indicating its potential to mitigate lipid peroxidation. The preclinical hepatoprotective effects of β-chitosan are ascribed to its antioxidant and antilipidemic properties [33]. Research has demonstrated the efficacy of β-chitin/chitosan against various tumour cells, including RT112 (urinary bladder carcinoma cell line) and HepG2. Cuttlefish-derived chitosan exhibited superior anticancer activity against human bladder cancer cells, with EC50 values of 62 μM and 50 μM after 48 and 72 hours of RT112 cell incubation, respectively. These findings suggest that β-chitosan, which forms low-viscosity solutions, inhibits tumour growth by enhancing T-cell proliferation, cytotoxic activity, and natural killer cell activity [48, 49]. While the antitumor potential of β-chitosan nanoparticles is compelling, the current evidence is largely restricted to cell culture and animal models. The specific toxicity profiles and biodistribution of β-chitosan in higher mammals are not fully understood. Transitioning from these promising animal results to human clinical trials remains a significant challenge.

7.6. Anticoagulant Activity

LMW sulphated β-chitosan derived from *S. pharaonis* has been found to exhibit marked anticoagulant activity in *in vitro* assays, highlighting its potential as a template for developing anticoagulant agents [50]. Research into the anticoagulant properties of β-chitosan has examined both the native polymer and its chemically modified derivatives. Native β-chitosan exhibits intrinsic anticoagulant activity; for example, Subhapradha *et al.* [51] investigated β-chitosan and reported significant prolongation of partial thromboplastin time (PTT) and prothrombin time (PT) in human plasma. Furthermore, chemical modification of β-chitosan through sulfation has been explored to enhance its anticoagulant efficacy. These sulphated derivatives are synthesised directly from β-chitosan obtained from cephalopod sources to mimic the structure of heparin. For instance, LMW sulphated β-chitosan derived from the cuttlefish *Sepia pharaonis* has shown significant anticoagulant potential [52]. Similarly, Sajith *et al.* [52] explored sulphated β-chitosan extracted from the internal bone of the spineless cuttlefish *Sepiella inermis*. Their study revealed that this sulphated β-chitosan exhibits heparin-like anticoagulant activity, as evidenced by its effects on activated partial thromboplastin time (APTT) and PT. Collectively, these findings suggest that β-chitosan serves as a versatile precursor for the development of novel anticoagulant agents, with sulfation offering a pathway to enhance therapeutic potential. However, the anticoagulant activity of sulphated β-chitosan is often compared to heparin. Although heparin's risk of inducing heparin-induced thrombocytopenia (HIT) is a well-known clinical issue, β-chitosan derivatives offer a potential alternative; their bleeding risk profiles and immunogenicity have not been characterised to the same extent as standard therapies. Moreover, the sulphation process must be carefully controlled to ensure batch-to-batch consistency, as process variability remains a major challenge in pharmaceutical development [51].

8. β-Chitosan in Nanocarriers

Chitosan is widely utilised as a pharmaceutical nanocarrier due to its cationic nature and mucoadhesive properties, which facilitate the binding of anionic drugs and enhance residence time at target sites. Beyond general safety considerations, the unique physicochemical properties of β-chitosan, such as its higher solubility and reactivity compared to α-chitosan, offer distinct advantages for nanoparticle synthesis. These characteristics enable efficient encapsulation of therapeutic agents and the formation of stable, controlled-release delivery systems [53, 54]. Its ability to form stable nanoparticles under mild conditions allows for the encapsulation and controlled release of therapeutic agents without compromising their bioactivity. The performance of β-chitosan nanocarriers is governed by key parameters, including MW and DDA. Optimising these factors allows for precise control over particle size, surface charge (zeta potential), and encapsulation efficiency. A prominent example of this application is the development of a delivery system for the enzyme β-galactosidase (β-gal). Researchers encapsulated the enzyme in LMW β-chitosan nanoparticles (β-CS NPs) stabilised with cellulose nanocrystals (CNC). This formulation demonstrated enhanced enzyme activity and controlled release, with 81.23% of β-gal activity retained after 2 hours. These findings suggest that β-chitosan-based nanocarriers are promising, non-toxic vectors for enzyme delivery and other therapeutic applications [55]. While the application of β-chitosan in nanocarriers demonstrates proof of concept, particularly for enzyme delivery, a major translational barrier is the stability of these nanoparticles under physiological conditions; their tendency to aggregate or dissolve across varying pH levels affects drug-release

kinetics. On top of that, scaling up the production of β-chitosan nanoparticles while maintaining monodispersity and high encapsulation efficiency presents a significant challenge. Regulatory approval for such novel marine-derived drug delivery systems also requires comprehensive characterisation of degradation products and long-term toxicity profiles [54].

9. β-Chitosan in Tissue Engineering Scaffolds

In tissue engineering, the utility of β-chitosan is largely defined by its capacity to form highly porous and interconnected three-dimensional matrices. The specific value of β-chitosan in this application lies in its superior solubility and reactivity compared to α-chitosan, which facilitates its processing into various scaffold architectures, including sponges, membranes, and nanofibers without compromising bioactivity (Figure 9) [56, 57]. Research has demonstrated the efficacy of β-chitosan-based scaffolds in bone regeneration. A hybrid scaffold composed of β-chitosan and gelatin, functionalized with recombinant human bone morphogenetic protein-2 (rhBMP-2), was found to significantly enhance cell viability and osteogenic potential, rendering it a promising biomaterial for bone repair [58].

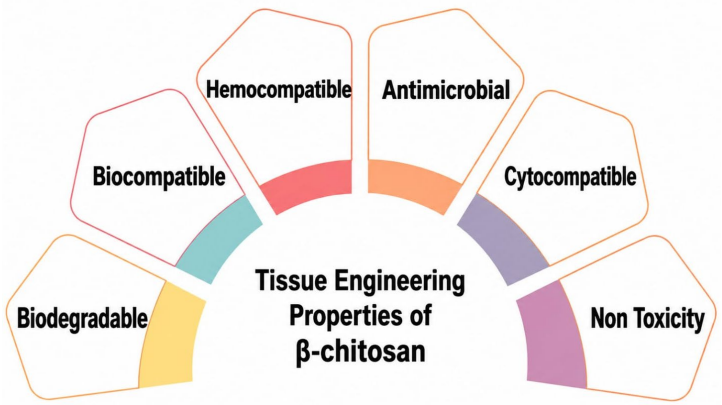

Figure 9. Tissue engineering properties of β-chitosan. This image was created with Edraw.AI

Furthermore, scaffolds fabricated from β-chitosan extracted from *Loligo duvauceli* (Indian squid) pens have shown promising structural and biological characteristics. These scaffolds had an average pore size of 60 µm and a swelling ratio of 16.2 ± 2.2%, providing a suitable environment for mouse fibroblast 3T3 cell adhesion and proliferation [13]. Recent advancements have also explored electrospun nanofibrous mats blending β-chitosan with poly(vinyl alcohol) (PVA). These nanofibrous scaffolds supported satisfactory levels of cell adhesion and viability, further confirming the potential of β-chitosan in advanced tissue engineering applications [59]. The fabrication of β-chitosan scaffolds shows considerable promise for regenerative medicine, but several limitations persist. Mechanical strength, a major requirement for load-bearing applications in hard tissue engineering, often remains insufficient for pure chitosan scaffolds, necessitating composite strategies. Furthermore, while *in vitro* biocompatibility is well established, the *in vivo* degradation rate of β-chitosan and the host inflammatory response during long implantation periods require further investigation. Further, translating these scaffolds into

clinical products demands the development of sterilisation methods that do not compromise the polymer's bioactivity or porous structure [60]. A summary of the biomedical applications, their advantages, limitations, and current levels of development for β-chitosan is provided in Table 2.

Table 2. Overview of biomedical applications, efficacy and limitations of β-chitosan.

Application area	Key advantages of β-chitosan	Limitations	Level of development	[Ref]
antibacterial agents	• superior positive charge density due to high DDA; • looser structure facilitates interaction with bacterial membranes; • effective against MDR strains (e.g., *S. aureus, P. aeruginosa*)	• efficacy varies based on extraction protocol (DDA/MW variability); • the ionic environment of physiological fluids may alter charge density	• *in vitro* (dominant); • *in vivo* (limited studies); • clinical: none	[14, 18, 35, 37–42]
antioxidant therapy	• high solubility allows efficient radical scavenging; • low MW fractions show enhanced activity via hydrogen donation	• antioxidant activity is generally lower than synthetic agents; • native MW (500–700 kDa) is less effective than depolymerised fractions	• *in vitro* (DPPH, hydroxyl radical assay); • *in vivo* (limited studies); • clinical: none	[13, 43, 44]
antitumor therapies	•immunoenhancement (T-cell and NK cell activation); • induction of apoptosis and antiangiogenic effects; • effective carrier for drug delivery (e.g., nanoparticles)	• biodistribution and toxicity profiles in higher mammals are not fully understood; • transition from animal models to humans is challenging	• *in vitro* (cell lines); • *in vivo* (rodent models); • clinical: none	[33, 46–49]
anticoagulants	• sulfated derivatives mimic heparin structure; • native polymer shows activity (prolongs PT/APTT); • potential alternative to heparin to avoid HIT	• bleeding risk and immunogenicity not fully characterised; • the sulphation process requires precise control for batch consistency	• *in vitro* (plasma assays: PT, APTT); • *in vivo* none; • clinical: none	[50–52]

Table 2. (continued) Overview of biomedical applications, efficacy and limitations of β-chitosan.

Application area	Key advantages of β-chitosan	Limitations	Level of development	[Ref]
nanocarriers (drug delivery)	• high solubility and reactivity aid in nanoparticle synthesis; • cationic nature allows encapsulation of anionic drugs (e.g., enzymes); • good mucoadhesive properties; • stability in physiological pH varies (risk of aggregation/dissolution)	• scaling up while maintaining monodispersity is difficult	• *in vitro* (proof-of-concept: β-gal delivery); • *in vivo* none; • clinical: none	[53–55]
tissue engineering scaffolds	• high porosity and water binding capacity support cell infiltration; • forms interconnected 3D matrices; • bood biocompatibility for fibroblast adhesion; • low mechanical strength (tensile and viscoelastic) compared to α-form	• unsuitable for load-bearing applications without composites; • sterilisation methods can damage the structure	• *in vitro* (cell culture studies); • *in vivo* limited; • clinical: none	[13, 32, 56–60]

10. Conclusions

In conclusion, this review highlights that β-chitosan is not just a variant of chitosan but a biopolymer with distinct structural advantages that offer superior performance in specific biomedical applications. Compared with the widely used α-chitosan, β-chitosan exhibits distinct benefits stemming from its unique parallel-chain alignment and loose crystalline packing. These structural features confer higher chemical reactivity, enhanced solubility, and a higher DDA, which altogether contribute to its promising antibacterial, antioxidant, and antitumor activities in preclinical studies. Furthermore, the extraction of β-chitosan from cephalopod sources presents a more environmentally friendly and cost-effective process, as it eliminates the need for the harsh demineralisation steps required for crustacean-derived α-chitosan. However, the practical application of β-chitosan is currently constrained by significant limitations compared to the established

α-form. The most crucial challenge is sourcing; while α-chitosan benefits from an abundant supply of crustacean shell waste, β-chitosan relies on cephalopod endoskeletons, which are less plentiful and subject to seasonal variability. This scarcity limits commercial availability and often results in higher costs. To fully realise the potential of β-chitosan, future research must move beyond efficacy reporting. Priority should be placed on (i) establishing industry-wide standards for β-chitosan purity and DDA; (ii) conducting advanced preclinical studies focused on safety and biodistribution; and (iii) developing robust supply chain strategies to ensure consistent raw material availability. Addressing these challenges is essential for β-chitosan to evolve into a viable commercial biomaterial.

11. Author Contributions

Conceptualisation, and design, R.A.B, S.R.; writing-original draft preparation, R.A.B, S.R.; manuscript writing, reviewing and editing, S.G, B.K, N.M.F. All authors have read and agreed to the published version of the manuscript.

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