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Chitosan as a haemostatic agent: Mechanisms, applications, safety, and future prospects in emergency bleeding control

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Abstract
Haemorrhage is a primary cause of mortality in traumatic injuries, underscoring the need for effective and rapidly deployable haemostatic agents. Chitosan, a natural polycationic biopolymer derived from chitin, has shown promise in controlling bleeding in emergencies. This review examines the essential properties of chitosan, including its biocompatibility, biodegradability, and antimicrobial activity. A key focus is its haemostatic mechanism, which includes the electrostatic aggregation of red blood cells and direct platelet activation, functioning independently of the classical coagulation pathway. This characteristic makes chitosan beneficial for patients with coagulopathies and those on anticoagulant therapy. Additionally, the review discusses the variety of chitosan-based products available, such as powders, sponges, and hydrogels, and their positive impact on patient survival rates. It also addresses critical physicochemical parameters influencing efficacy, such as molecular weight and degree of deacetylation. Finally, current challenges, including solubility and mechanical strength, are identified, along with future directions for developing advanced nanocomposites and optimized biomaterials to enhance their clinical utility in emergency medicine.

Keywords: Chitosan, haemostasis, bleeding, haemostatic agents, biomaterials, coagulation

Introduction
Uncontrolled haemorrhage presents a significant challenge in emergency medicine and is a leading cause of preventable death in both civilian and combat trauma. Approximately 35–40% of these fatalities occur in pre-hospital settings due to massive blood loss. The situation is further complicated by an increasing number of patients undergoing anticoagulant therapies, which impair physiological clotting and render many traditional haemostatic agents less effective. This critical unmet need highlights the urgency for developing safe, effective, and rapidly deployable haemostatic materials that can work effectively regardless of a patient's coagulation status.

Chitosan has gained significant attention as a leading hemostatic agent among various biomaterials. It is a natural, polycationic polysaccharide derived from the deacetylation of chitin, which is the second most abundant biopolymer in nature, following cellulose. Sourced renewably from crustacean shells and fungi, chitosan offers a unique combination of beneficial properties, including excellent biocompatibility, biodegradability, non-toxicity, and inherent antimicrobial activity. The primary value of chitosan in haemostasis lies in its cationic nature, which enables a rapid and complex mechanism of action that often functions independently of the classical coagulation cascade. This review aims to provide a comprehensive overview of chitosan as a haemostatic agent by detailing its fundamental properties, elucidating its mechanisms of action, summarizing its clinical applications and safety profile, and discussing the challenges and prospects for its use in emergency bleeding control.

Structure and Fundamental Properties of Chitosan
Chitosan is a linear polysaccharide made up of randomly distributed β-(1→4)-linked D-glucosamine and N-acetyl-D-glucosamine units. It is derived from chitin through the chemical or enzymatic hydrolysis of acetyl groups under alkaline conditions.

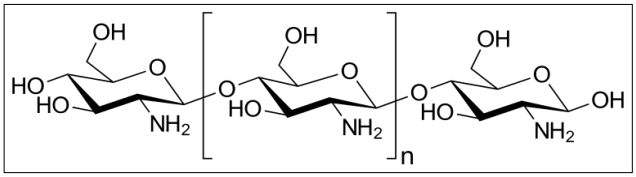

Fig 1: Chemical structure of Chitosan: β-(1→4)-linked D-glucosamine and N-acetyl-D-glucosamine

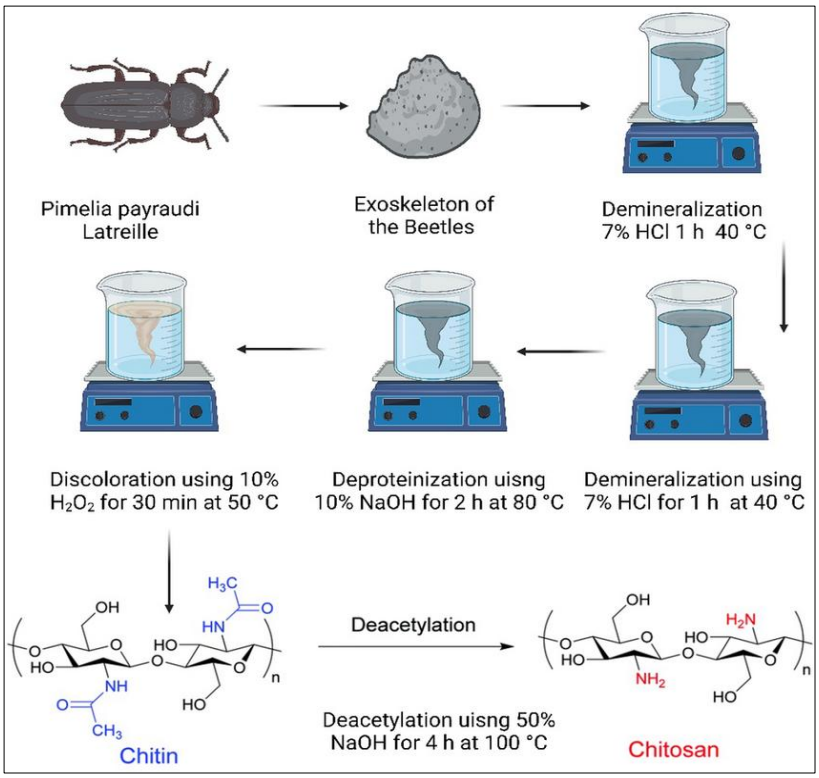

Fig 2: Derivation of Chitosan from the exoskeleton of *Pimelia payraudi*

The degree of deacetylation (DD), which refers to the percentage of D-glucosamine units, is the critical parameter that differentiates chitosan from chitin and fundamentally influences its properties. Typically, a DD of 60% or higher is required for a polymer to be classified as chitosan.

Chitosan's usefulness in biomedical applications stems from several key properties:

1. **Biocompatibility and Biodegradability:** Chitosan is well-known for its excellent biocompatibility and low toxicity. Its degradation products are benign amino sugars that are easily absorbed and metabolized by human tissues, leaving no harmful residues.
2. **Polycationic Nature:** The primary amine groups ($-NH_2$) on the D-glucosamine units impart a polycationic character to chitosan. With a pKa of approximately 6.5, these amine groups become protonated ($-NH_3^+$) in acidic to neutral solutions, resulting in a positive surface charge. This positive charge is essential for its haemostatic and mucoadhesive properties.
3. **Antimicrobial Activity:** Chitosan exhibits broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as fungi. This effectiveness is largely due to the electrostatic interactions between its protonated amino groups and the negatively charged components of microbial cell membranes, leading to cell disruption and death.
4. **Processability:** Chitosan can be easily processed into various forms, including powders, membranes, sponges, nanofibers, and hydrogels. This versatility allows for the development of customized products suited for different types of wounds and clinical scenarios.

Despite these advantages, native chitosan has some limitations, primarily its poor solubility in water and neutral pH solutions, as well as its inadequate mechanical strength, which can restrict its applications.

Table 1: Key Properties and Forms of Chitosan

Characteristic	Description
Definition	Natural biopolymer derived from chitin, the second most abundant biopolymer.
Chemical Structure	N-deacetylated derivative of chitin; 2-amino-2-deoxy-β-D-glucopyranose units. Contains primary/secondary hydroxyl and amine groups.
Key Properties	Biodegradability, Biocompatibility, Polycationic Nature, Haemostatic Action, Antimicrobial Activity, Low Toxicity.
Influence of DD & MW on Solubility	Higher DD% increases water solubility due to more protonated amino groups. Higher MW decreases solubility due to increased H-bonding.
Common Processed Forms	Membranes, sponges, gels, scaffolds, microparticles, nanoparticles, nanofibers, 3D printed constructs.
Inherent Limitations	Poor solubility in water/organic solvents (due to H-bonding), compromising mechanical strength.

Mechanisms of Chitosan-Mediated Haemostasis

The haemostatic effectiveness of chitosan is due to a complex mechanism that often functions independently of the traditional coagulation cascade, offering a significant advantage in critical bleeding situations.

1. Elucidation of the Non-Classical Coagulation Pathway

A key feature of chitosan's haemostatic action is its ability to promote coagulation independently of the intrinsic pathway of the classical coagulation cascade, which is typically responsible for fibrin formation. This independence is especially beneficial in clinical situations where fibrin production is impaired, such as in patients receiving heparin or those with other clotting disorders. Chitosan's ability to form a stable clot with blood, even in the absence of fibrin, makes it a crucial backup haemostatic agent when normal clotting mechanisms are compromised. This unique characteristic is life-saving for certain patient populations and offers a distinct advantage over traditional agents that depend on a fully operational coagulation cascade.

2. Direct Interaction with Red Blood Cells (RBCs)

Chitosan contains positively charged amine groups (-NH3+) that interact electrostatically with the negatively charged proteins and glycolipids found on the surface of red blood cell (RBC) membranes. This charge-mediated interaction leads to the aggregation and clumping of RBCs around a wound site, creating an immediate physical barrier to blood loss.

The polymer chains of chitosan can effectively crosslink erythrocytes, forming a lattice-like structure that physically traps blood cells, thus creating an artificial clot. Additionally, contact with chitosan can induce morphological changes in RBCs, causing them to lose their characteristic biconcave shape and develop an unusual affinity for one another, which further contributes to their aggregation.

Studies indicate that low molecular weight chitosan can directly bind to erythrocyte membranes, playing a significant role in hemagglutination. The effectiveness of chitosan in initiating coagulation through this mechanism is closely related to its degree of deacetylation (DD), as it is dependent on the number of protonated amine groups available for interaction. Moreover, the interaction with RBCs tends to increase with higher molecular weight chitosan, likely due to greater polymer chain entanglement, which facilitates more extensive crosslinking.

This direct, charge-mediated clumping of RBCs by chitosan provides an immediate physical barrier to blood loss, acting as a rapid "first responder" mechanism that is independent of the slower enzymatic cascade involved in clotting.

3. Stimulation of Platelet Adhesion, Aggregation, and Activation

Chitosan plays a vital role not only in its direct effects on red blood cells (RBCs) but also in stimulating platelet adhesion, aggregation, and activation. Research has shown that chitosan films can induce platelet adhesion and aggregation, as well as activate the intrinsic blood coagulation pathway. Platelets exhibit stronger attachment to chitin and chitosan particles, forming aggregated masses with elongated projections. A high positive charge density in chitosan is crucial for enhancing both platelet adhesion and aggregation.

Recent studies have revealed a more complex and direct involvement of chitosan in physiological clotting than previously understood. It has been demonstrated that chitosan can trigger intracellular signalling reactions that activate glycoprotein IIb/IIIa and promote the release of thromboxane A2 and ADP. These signals contribute to increased platelet spreading and enhance the stability of adhesion at the wound site. Notably, recent findings suggest that chitosan can induce coagulation by directly activating platelet Toll-like receptor 2. This discovery challenges earlier assumptions that chitosan does not directly influence physiological clotting mechanisms, paving the way for targeted modifications of chitosan aimed at improving platelet-mediated haemostasis. Identifying this direct activation pathway indicates a more integrated and complex role for chitosan in the coagulation process, potentially leading to the development of more effective haemostatic agents.

4. The Role of Plasma Sorption and Concentration of Blood Components at the Wound Site

Chitosan's haemostatic action is enhanced by its ability to absorb plasma. It can absorb a considerable amount of fluid, ranging from 50% to 300% of its dry weight, resulting in a localized concentration of red blood cells (erythrocytes) and platelets at the site of injury. This plasma absorption is crucial for chitosan's effectiveness in stopping bleeding, with factors such as molecular weight, degree of deacetylation (DD), and the specific type of chitosan material affecting its absorption rate.

Water molecules readily bind to the active sites of polysaccharides, and the sorption rate increases with a higher degree of deacetylation. Although plasma absorption alone may not be the main factor in halting bleeding, it plays a significant role in concentrating blood cells, which facilitates the subsequent cellular and molecular interactions essential for clot formation.

Additionally, chitosan can absorb fibrinogen and other plasma proteins, thereby further promoting erythrocyte adhesion and coagulation in chitosan solutions. The combined effects of plasma absorption and direct cellular interactions—such as red blood cell agglutination and platelet activation—create a synergistic haemostatic effect. This initial physical concentration of blood components prepares the environment for more efficient cellular and molecular interactions, resulting in rapid and strong clot formation.

5. Impact of Chitosan's Positive Charge Density and Molecular Characteristics on Haemostatic Efficacy

The positive charge of chitosan is essential for its electrostatic interactions with the negatively charged components of blood. The degree of protonation of its amino groups, which is directly influenced by the degree of deacetylation (DD), plays a crucial role in its ability to adsorb red blood cells and initiate coagulation. Additionally, a higher molecular weight of chitosan usually leads to increased interaction with red blood cells, likely due to enhanced polymer chain entanglement and greater crosslinking. These molecular characteristics highlight the importance of precise material engineering to optimize the haemostatic performance of chitosan.

Table 2: Proposed Mechanisms of Chitosan Haemostasis

Mechanism	Description	Key Interactions/Features	Impact on Haemostasis
Non-Classical Pathway	Triggers coagulation independently of the classical coagulation cascade (fibrin formation).	Operates in the absence of fibrin; useful when physiological clotting is inhibited (e.g., by heparin, coagulopathies).	Provides a distinct advantage over traditional agents; functions as a critical "backup" haemostat.
Red Blood Cell (RBC) Aggregation	Positively charged chitosan electrostatically interacts with negatively charged RBC membranes.	-NH3+ groups bind to RBCs; crosslinking of erythrocytes; changes in RBC morphology (loss of biconcave shape); influence of DD and MW.	Forms an immediate physical barrier; rapid and independent of enzymatic cascade.
Platelet Activation	Induces adhesion, aggregation, and activation of platelets.	Platelets attach strongly to chitosan; high positive charge density is crucial; activates glycoprotein IIb/IIIa; discharges thromboxane A2/ADP; direct activation of platelet Toll-like receptor 2.	Accelerates clot formation; strengthens adhesion stability; suggests direct involvement in physiological clotting.
Plasma Sorption/Concentration	Absorbs liquid from plasma, leading to a localized concentration of blood components.	Absorbs 50-300% of its weight; concentrates erythrocytes and platelets; absorbs fibrinogen and other plasma proteins	Creates a synergistic effect by priming the environment for cellular and molecular interactions.

4. Clinical Applications in Emergency Bleeding and Haemorrhage Control:

Chitosan possesses versatile properties that facilitate its formulation into various forms, rendering it exceptionally adaptable for clinical applications, particularly in the management of emergency bleeding and hemorrhage control.

4.1. Various Forms of Chitosan-Based Haemostatic Products

Chitosan can be processed into various forms, including membranes, sponges, gels, scaffolds, microparticles, nanoparticles, nanofibers, and 3D printed constructs. For haemostatic applications, common forms include powders, bandages, sponges, and hydrogels. Powder formulations are particularly appreciated for their ease of use, as they can easily conform to the irregular shapes of wounds, making them simpler to apply in emergencies. The versatility of chitosan allows for the creation of customized product designs that effectively address different types of wounds and bleeding scenarios. For example, powders are ideal for irregular or deep wounds, while bandages are better suited for larger surface areas, thereby enhancing overall clinical effectiveness.

4.2. Advantages in Emergency Settings

Chitosan-based haemostatic agents offer several significant advantages in emergency settings:

- **Immediate Action:** Chitosan powder is engineered to initiate clotting upon direct contact with blood, rapidly forming a stable seal over the wound site to staunch bleeding within a minute.
- **Ease of Application:** Products such as Chitosan Powder are designed to be user-friendly, making them accessible to both trained medical professionals and individuals in critical emergencies.
- **Versatility:** Chitosan-based products offer a versatile solution for a wide range of injuries. They are effective for managing minor cuts and abrasions, as well as severe lacerations and punctures, and are specifically indicated for both arterial and venous bleeding, as well as moderate to severe hemorrhage.
- **Physical Mechanism:** The haemostatic action of chitosan operates physically. It is absorbed into body fluids in powder form, creating a gel-like structure that tightly binds powder granules to stop bleeding, without interfering with the body's natural systemic coagulation processes
- **Safety Profile:** These products have undergone extensive safety testing and are reported to be non-allergenic and non-heat-producing upon application.

Chitosan stands out as a potentially life-saving first-line haemostat due to its rapid action, ease of use, and effectiveness across various types of bleeding. This is especially important in pre-hospital and tactical environments, where resources may be limited and situations can be chaotic. The ability to quickly and easily control bleeding can significantly impact patient survival in these critical moments.

4.3. Reported Impact on Patient Outcomes

The benefits of chitosan extend beyond immediate haemostasis, contributing significantly to improved patient outcomes:

- **Reduced Complications:** Chitosan-based agents promote faster clotting and minimize blood loss, which helps reduce the risk of common complications such as infection, shock, and delayed healing. Additionally, chitosan's inherent antibacterial properties play a significant role in preventing wound infections.
- **Enhanced Emergency Response:** These agents provide first responders with a reliable tool to quickly control bleeding, significantly improving the chances of survival and recovery for patients in critical situations.
- **Decreased Mortality and Increased Survival:** Chitosan-based products are specifically designed to reduce mortality rates associated with blood loss and to increase survival rates in cases of severe bleeding and uncontrolled haemorrhage.
- **Clinical Evidence:** Research using animal models has demonstrated the effectiveness of chitosan-based dressings in reducing haemorrhage and improving survival rates. Furthermore, early clinical experiences from combat operations, particularly with the HemCon dressing, have validated its haemostatic efficacy in real-world scenarios.

Chitosan offers a variety of benefits that go beyond just stopping bleeding. It has antimicrobial properties and helps reduce secondary complications, which can significantly enhance a patient's overall prognosis. Its advantages extend beyond initial control of bleeding to promoting a better long-term recovery. By preventing infections and shock, chitosan directly influences the patient's healing process.

5. Safety Profile and Toxicological Considerations

It is essential to understand the safety profile of chitosan for its effective application in emergency haemostasis within the biomedical field.

5.1. Summary of Human Tolerance Studies for Chitosan

Studies on humans regarding chitosan, mainly as a weight-loss supplement, indicate that it is generally well tolerated. No adverse effects were reported among male volunteers who took 4.5 g/day of chitosan orally for 12 days, nor among female volunteers who received 2.5 g/day for the same duration. Furthermore, the administration of chitosan at doses up to 6.75 g/day for 8 weeks also showed no reported adverse effects in human volunteers. The observed tolerance to oral chitosan, even at relatively high doses for weight loss, suggests a favorable safety profile for internal exposure. This contributes to our understanding of its systemic toxicity, which is relevant even for materials primarily used topically, due to the potential for some systemic absorption, especially in cases of extensive wound application.

5.2. Detailed Analysis of Animal Toxicity Studies (NTP Study)

The National Toxicology Program (NTP) conducted a comprehensive 6-month feeding study using Sprague-Dawley rats to assess the effects of dietary chitosan on several physiological parameters. These parameters included bone metabolism, levels of fat-soluble vitamins, the absorption of dietary fat and calcium, and general toxicity.

Study Design: Rats were fed diets containing 0%, 1%, 3%, or 9% chitosan for 25 to 26 weeks. The average daily doses administered ranged from approximately 450 mg/kg/day to 5,200 mg/kg/day for male rats and from 650 mg/kg/day to 6,000 mg/kg/day for female rats. The AIN-93M diet, which features lower fat and fat-soluble vitamin content, was specifically selected for this study to avoid any confounding results that might arise from potential vitamin depletion.

Observed Effects

Fat Digestion: Significant, dose-dependent reductions in the percentage of fat digested were observed. In 9% of males, a decrease of 20-33% was noted, while 9% of females showed a decrease of 5-14%. Additionally, effects were also observed in 3% of males, who experienced a 2-8% reduction in fat digestion. These changes resulted in a corresponding increase in both fecal weight and moisture.

Vitamin Levels

- **Serum Vitamin A:** Significant decreases were observed in 9% of males, with a decrease ranging from 26% to 29%, and in 3% of males, with a decrease of 15% to 16%. Additionally, 9% of females also experienced decreases of 18% to 21%.
- **Serum Vitamin E:** Substantial reductions were noted in various groups: 3% of males showed a decrease of 33% to 42%, while 9% of males experienced a much larger decrease of 79% to 82%. Furthermore, 1% of males had a 17% decrease. In females, 9% exhibited significant reductions of approximately 60%.
- **Hepatic Vitamin E:** Significant reductions were noted in 3% of males with a decrease of 48% and in 9% of males with an 87% decrease. Similarly, 9% of females experienced an 80% decrease.
- **Serum 1,25 (OH)2 Vitamin D:** Significant increases were found in 9% of males, with an increase ranging from 105% to 142%, as well as in females, who showed an increase of 100% to 180%. This elevation may be a compensatory response to the observed low phosphorus levels.
- **Organ Weights:** Both absolute and relative liver weights in males and females decreased significantly by 21-22% in the 9% group compared to controls. Similarly, absolute and relative thymus weights were significantly reduced in males at both 3% and 9%, as well as in females at 9%.
- **Liver Histology:** A treatment-related decrease in the incidence of periportal fatty change was observed in the livers of 9% of females, with a non-significant decrease also noted in 9% of males. This histological change is considered an adaptive response to the observed depletion of vitamins and minerals.
- **Bone Parameters:** The study's findings did not support the idea that chitosan causes bone resorption. Although there were occasional and inconsistent increases in parathyroid hormone levels, calcium levels remained relatively stable. Moreover, assessments of bone calcium content, bone length, and histological analysis of bone tissue did not show any evidence of calcium loss from the bone after exposure to chitosan.
- **Other Clinical Findings:** No treatment-related clinical findings were observed in the core study animals. However, 13 animals from the special study groups B and C, mostly from the 9% chitosan group, experienced seizures during or after the 18-week blood collection period. The cause of these seizures remains undetermined. Additionally, five rats from these groups died, often following a seizure.

Lowest-Observed-Effect Level (LOEL): Based on these results, the LOEL for chitosan exposure was determined to be 1% (approximately 450 mg/kg) in male rats and 9% (approximately 6,000 mg/kg) in female rats.

The NTP study indicates that while chitosan is generally safe for topical use, high oral doses can lead to significant nutritional deficiencies. These deficiencies especially impact fat-soluble vitamins A and E, as well as phosphorus, and there may be noticeable changes in organ weights. Although the cause of the observed seizures was not determined, they raise concerns about potential neurological effects at high exposure levels. This emphasizes the importance of considering the route of administration and the possibility of systemic absorption, even for substances primarily used topically. This is particularly relevant when chitosan is applied to large or compromised wound areas, where absorption may be increased.

5.3. Discussion of Potential Adverse Effects and Their Clinical Relevance

The occurrence of seizures in some animal models, especially at higher doses of chitosan, is a significant adverse effect. However, the direct causal link to chitosan and the underlying mechanism are still not well understood. For topical haemostatic agents, it is crucial to consider the risk of systemic absorption and potential adverse effects, particularly when large quantities are applied or in vulnerable populations, such as paediatric patients. The observation of seizures in animal models, despite the lack of a definitive cause, highlights the need for rigorous post-market surveillance and further targeted research. This is essential to thoroughly

investigate and, if possible, rule out neurotoxicity, especially if systemic absorption could occur in clinical use.

5.4. Confirmation of Non-Allergenic and Non-Heat Producing Properties for Topical Formulations

An important safety feature of chitosan for direct wound application is that it is both non-allergenic and does not produce heat. Commercial products like Clotsan Powder have been thoroughly tested for safety and are confirmed to generate "no heat and are non-allergenic." This addresses two common concerns related to wound dressings: the risk of local irritation or tissue damage from exothermic reactions and the possibility of allergic reactions. These attributes greatly improve patient comfort and safety during application.

Table 3: Summary of Chitosan Toxicity Findings in Animal Models (NTP Study)

Parameter	Male Rats (LOEL: 1% / 450 mg/kg/day)	Female Rats (LOEL: 9% / 6,000 mg/kg/day)	Implications
Fat Digestion (%)	Decreased (2-8% at 3%; 20-33% at 9%)	Decreased (5-14% at 9%)	Reduced fat absorption, increased fecal weight/moisture.
Serum Vitamin A	Decreased (15-16% at 3%; 26-29% at 9%)	Decreased (18-21% at 9%)	Potential nutritional inadequacy, especially long-term.
Serum Vitamin E	Decreased (17% at 1%; 33-42% at 3%; 79-82% at 9%)	Decreased (approx. 60% at 9%)	Significant fat-soluble vitamin depletion.
Hepatic Vitamin E	Decreased (48% at 3%; 87% at 9%)	Decreased (80% at 9%)	Significant fat-soluble vitamin depletion in liver.
Serum 1,25 (OH)2 Vitamin D	Increased (105-142% at 9%)	Increased (100-180% at 9%)	Compensatory response to phosphorus depletion.
Phosphorus	Decreased (12-18% at 9%; 14% ; 3%	Decreased (16-20% at 3%; 9-15% at 9%)	Mineral imbalance, potential impact on bone health
Cholesterol & Triglycerides	Decreased (26-48% cholesterol at 9%; 47-57% triglycerides at 9%)	Decreased (26-48% cholesterol at 9%; 30% triglycerides at 9%)	Cholesterol-lowering effect, consistent with other studies.
Liver Weights (Absolute & Relative)	Decreased (22% lower at 9%)	Decreased (21% lower at 9%)	Loss of fat accumulation in liver.
Thymus Weights (Absolute & Relative)	Decreased (at 3% and 9%)	Decreased (at 9%)	Suggests nutritional deficiencies or stress response.
Liver Histology	Decreased incidence/severity of periportal fatty change (at 1%, 3%, 9%)	Decreased incidence of periportal fatty change (at 1%, 3%, 9%)	Adaptive response to vitamin/mineral depletion.
Bone Parameters	Unaffected	Unaffected	No evidence of bone resorption.
Seizures (Special Study Groups)	Observed (2 at 1%; 1 at 3%; 10 at 9%)	Observed (predominantly at 9%)	Cause undetermined; warrants further investigation into neurological effects.

6. Commercial Chitosan-Based Haemostatic Products

The effectiveness of chitosan as a haemostatic agent is well-established, with various commercial products available worldwide. These products utilize chitosan's unique properties to effectively control bleeding in different formulations.

6.1. Identification and Characterization of Key Market Products:

- HemCon® Haemostatic Bandages**:** These are some of the most recognized chitosan-coated bandages, commonly used for managing external haemorrhage in military operations and pre-hospital bleeding emergencies. HemCon® bandages are FDA-approved for hemorrhage control. Their design features a freeze-dried chitosan-based dressing that is optimized for mucoadhesive surface density and structural integrity at the injury site. They are typically available as square dressings measuring 10 cm × 10 cm and approximately 2 mm thick, with a nonabsorbable backing, packaged in a vacuum-sealed aluminum pouch.
- GuardaCare®, ChitoFlex®, and ChitoGauze®: These products are from the same company that produces HemCon®. GuardaCare® and ChitoFlex® are designed to function as temporary surgical dressings and stuffable dressings, respectively. ChitoGauze® is a medical gauze made from a non-woven blend of polyester and rayon, coated with chitosan. All of these products aim to provide an antibacterial barrier along with their hemostatic properties.
- Celox™™ Gauze:This is a chitosan-coated haemostatic gauze that has proven effective for controlling bleeding in emergencies. Celox is also available in a powder formulation, adding versatility to its application.
- Other Chitosan-Containing Wound-Care Products: The market also includes products like ChiGel, Chitopack C®, and TraumaStat.

The commercial success and widespread adoption of chitosan-based haemostats, particularly within military operations, underscore their real-world efficacy and practicality. This transition from a research material to a clinically indispensable tool validates chitosan's significant role in high-stakes medical interventions.

(Table 4: Overview of Commercial Chitosan-Based Haemostatic Products)

Product Name	Company	Form	Key Features	Regulatory Status
HemCon® Bandage	HemCon Medical Technology Inc	Lyophilized (porous) sterile bandage	Haemostatic, antimicrobial, and optimizes mucoadhesive surface density.	FDA Approved
GuardaCare® XR Surgical	HemCon Medical Technology Inc.	Temporary surgical dressing	Haemostatic, antimicrobial.	FDA Approved

ChitoFlex® PRO	HemCon Medical Technology Inc.	Stuffable dressing	Haemostatic, antimicrobial.	FDA Approved
ChitoGauze® XR PRO	HemCon Medical Technology Inc.	Non-woven polyester-rayon gauze	Haemostatic, antimicrobial, suitable for complex wound geometry.	FDA Approved
Celox™™ Gauze	Med Trade Product PVT LTD UK	Gauze, also a powder formulation	Stops severe bleeding, especially tactical combat casualties.	FDA Approved
Chitopack C®	Eisai Co.	Cotton-like dressing	Rebuilding normal subcutaneous tissue, regeneration.	Not approved
TraumaStat	Not specified	Not specified	Wound care.	Not specified
ChitoSAM 100	SAM Medical	High-performance dressing	Haemostatic, designed to stop bleeding fast.	Not approved
AxioStat	Axio Biosolutions Pvt LTD	Dressing	Stops bleeding instantly.	FDA Approved
MaxioCel	Axio Biosolutions Pvt LTD	Dressing	Treats pressure sores, diabetic foot ulcers, abrasions, burns, and surgical wounds.	FDA Approved

7. Challenges and Future Directions in Chitosan Hemostasis

Despite its significant advancements and established clinical utility, chitosan still faces inherent limitations that present ongoing challenges and drive future research directions in haemostasis.

7.1. Strategies to Overcome Current Limitations

The main factors limiting the wider biomedical application of chitosan are its poor solubility in aqueous solutions, primarily due to its high molecular weight and extensive hydrogen bonding, as well as its inferior mechanical properties. To overcome these limitations, researchers are utilizing various strategies:

Chemical Modifications: The reactive functional groups in chitosan, specifically the hydroxyl (-OH) and amine (-NH2) groups, provide numerous sites for chemical modification. These modifications can significantly enhance solubility, biocompatibility, adsorption properties, and mechanical strength. Examples include

- **Carboxymethyl Chitosan (CMC):** CMC derivatives, formed by replacing free amino and hydroxyl groups with carboxyl functional groups, exhibit good water solubility and enhanced biological properties.
- **N-Alkylation:** Introducing alkyl groups, such as N-octadecyl CS, can improve water solubility and impart hydrophobic properties that significantly enhance red blood cell aggregation and coagulation.
- **Quaternization:** This modification adds quaternary ammonium groups to the amino groups of chitosan, resulting in enhanced solubility in both acidic and basic environments, as well as improved mucoadhesive characteristics.
- **Graft Copolymerization:** Attaching different polymers to the chitosan backbone can improve water solubility, stability, muco-adhesion, and antibacterial properties.

Nanocomposites: Combining chitosan with other polymers, metals, or nanofillers is an effective strategy to enhance its properties. The incorporation of metallic nanoparticles, such as silver (Ag), copper (Cu), and zinc oxide (ZnO), along with graphene oxide (GO), cellulose nanocrystals (CNC), polyaniline, Montmorillonite, or hydroxyapatite, can significantly improve mechanical strength, thermal stability, electrical conductivity, and antimicrobial properties. Moreover, chitosan can serve as a reducing agent for metallic nanoparticles, which may help mitigate the cytotoxicity of these metallic components.

The ongoing pursuit of chemical modifications and the development of nanocomposites represents a shift from merely utilizing chitosan's natural properties to engineering "designer" haemostats with optimized performance. This approach enables the addressing of specific clinical requirements, such as injectability, self-healing capabilities, and extended stability, thereby enhancing its clinical utility.

7.2. Emerging Research Areas

Future research in chitosan haemostasis is focusing on developing more sophisticated and responsive materials:

- **Optimizing Material Architecture:** Researchers are drawing inspiration from the biological structure of lung alveoli to design haemostatic agents with optimized pore size and connectivity. This detailed architectural design aims to facilitate rapid blood absorption and promote the localized concentration of platelets and fibrin microthrombi, thereby enhancing the efficiency of clotting.
- **Exploring Novel Platelet Activation Pathways:** Further investigation into newly identified mechanisms, such as the direct activation of platelet Toll-like receptor 2 by chitosan, is an active area of current research. This deeper understanding of molecular interactions may lead to more targeted and effective haemostatic agents.
- **Injectable and Self-Healing Hydrogels:** The creation of chitosan-based hydrogels with fast gelation rates, adequate adhesion, and excellent mechanical properties is essential. These hydrogels can effectively seal irregularly shaped wounds through minimally invasive techniques, providing significant benefits in complex trauma situations.
- **Easy Removability:** An important factor for future haemostats is their easy removal from the wound site after achieving initial haemostasis, ensuring they do not cause further tissue damage or leave toxic residues.
- **Minimizing Cytotoxicity:** Although chitosan is generally regarded as non-toxic, further research is needed to address potential cytotoxic effects, especially for new nanocomposite formulations used in wound dressings.
- **Systematic Safety Assessment:** A systematic method for assessing the selectivity of drug delivery systems, performing comprehensive in vitro and in vivo toxicity studies, and addressing the safety concerns of chitosan-based biomaterials and their synthesis methods is essential.

The emphasis on advanced architectural design, targeted molecular interactions, and dynamic material properties—such as injectability and self-healing—signals a significant shift towards highly sophisticated, responsive chitosan-based haemostats. These innovative materials are being specifically designed for precision medicine and complex surgical procedures, representing the next generation of haemostatic solutions.

7.3. The Imperative for Comprehensive In Vitro and In Vivo Toxicity and Safety Assessments

Despite the submission of thousands of research studies and promising results from in vitro experiments, the widespread clinical application of chitosan in the biomedical field remains limited. Many unresolved issues and challenges need to be addressed to develop more advanced composites suitable for human clinical use. A thorough investigation into the toxicity of these novel nanocomposites is essential. Historically, research focused primarily on material fabrication and in vitro evaluations. However, there is now a growing emphasis on conducting more rigorous in vivo studies, despite the challenges this entails. The gap between encouraging laboratory results and successful clinical applications for complex chitosan composites highlights the need for robust, long-term in vivo studies and the establishment of standardized safety protocols. These steps are crucial for ensuring clinical utility and facilitating the regulatory approval of future chitosan-based haemostatic agents.

Table 5: Strategies for Enhancing Chitosan Properties for Haemostasis)

Limitation	Strategy	Specific Examples	Enhanced Property/Benefit
Poor Solubility	Chemical Modification	Carboxymethyl Chitosan (CMC), N-Alkylation, Quaternization, Graft Copolymerization	Improved water solubility, amphoteric nature, and enhanced muco-adhesion.
Inferior Mechanical Strength	Chemical Modification, Nanocomposite Formation	Cross-linking, incorporation of metallic nanoparticles (Ag, Cu, ZnO), Graphene Oxide (GO), Cellulose Nanocrystals (CNC), other polymers/nanofillers.	Enhanced mechanical strength, tunable properties, and increased stability.
Moderate Antibacterial Properties	Nanocomposite Formation	Combination with metallic nanoparticles (Ag, Cu, ZnO), Graphene Oxide (GO).	Stronger antibacterial activity against a broad spectrum of microbes, and reduced cytotoxicity of metals.
Limited Application in Irregular Wounds	Advanced Material Design	Injectable hydrogels, self-healing materials.	Rapid gelation, sufficient adhesion, ability to fill irregular shapes, and minimally invasive application.
Potential Cytotoxicity (for new composites)	Nanocomposite Formation	Chitosan as a reducing agent for metallic nanoparticles.	Reduced cytotoxicity of incorporated components.

8. Conclusion

Chitosan is a natural biopolymer with positive properties that play a crucial role in modern bleeding control, or haemostasis. Its key advantages include biodegradability, biocompatibility, and the ability to function independently of the classical coagulation cascade, making it a promising haemostatic agent. Chitosan can quickly agglutinate red blood cells, activate platelets directly, and effectively absorb plasma at the wound site, all of which contribute to rapid and effective bleeding control.

Chitosan's versatility allows it to be formulated into various product forms, such as powders, bandages, and hydrogels, making it suitable for many emergency bleeding situations. These formulations are designed for immediate action and ease of use, which are critical in time-sensitive scenarios. Their application significantly reduces complications like infection and shock, ultimately improving survival rates in essential events of bleeding. Although high oral doses in animal studies have indicated potential nutritional impacts and some adverse effects, such as seizures, topical formulations generally have a favourable safety profile; they are non-allergenic and do not produce heat.

The field of chitosan haemostasis is dynamic, with ongoing scientific efforts to overcome challenges like poor solubility and mechanical strength. This is being achieved through advanced chemical modifications and the development of sophisticated nanocomposites. These innovations are not mere incremental advancements but represent a strategic move towards creating "designer" biomaterials with enhanced performance and novel functionalities. The future of chitosan in emergency medical interventions looks exceptionally promising, with research aiming for highly responsive, injectable, and self-healing haemostats tailored for precise application in complex trauma and surgical contexts. Continued rigorous safety assessments, both in vitro and in vivo, are essential for successfully translating these cutting-edge innovations into widely adopted clinical solutions, ultimately saving more lives in critical bleeding situations worldwide.

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