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Review Article

A glimpse on the function of chitosan as a dental hemostatic agent

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SUMMARY

Managing a bleeding patient can be a challenge during dental surgery. Profuse hemorrhage due to platelet defects, coagulation disorders, vascular anomalies, medication-induced patients, as well as inherited bleeding ailments result in soft tissue hematoma, septic shock, compromised airway, and in some severe cases, death could occur. A vast array of surgical hemostatic agents are available to stop bleeding, including chitosan-based hemostatic agents. Chitosan has an advantage over other topical hemostatic materials for its ability to promote shorter bleeding times and assist in healing. Massive behind-the-scene research and development efforts are ongoing to increase the performance of chitosan as a hemostatic agent. Numerous studies on chitosan use in dental hemostasis have registered it as being safe, biodegradable, biocompatible, promoting healing, antimicrobial and bioactive. This article reviews the application of chitosan in managing hemostasis in dental patients.

1. Introduction

Chitosan is a biopolymer that is made up of β-(1,4)-linked D-glucosamine and N-acetyl-D-glucosamine units resulting from the deacetylation of chitin [1,2]. At least 60% of deacetylated chitin should consist of D-glucosamine residues to be classified as chitosan [3]. Crustaceans such as crabs, lobsters, prawns, shrimps, and insects have outer shells/exoskeletons which are primary sources of chitin [2,4]. Chitin is soluble in many inorganic and organic acids, such as acetic, lactic, malic, formic, and succinic acids, but is insoluble in most organic solvents [5] and aqueous solutions of pH > 7. Chitosan is polycationic at pH < 6 due to the protonation of its amino group and interacts readily with anionic polysaccharides, forming polyelectrolyte complexes [2,6].

Chitosan generally has a molecular weight of between 200–1000 kDa, based on its source and method of extraction [7]. The method used for extraction of chitosan varies between sources, as compositions vary between sources. Table 1 below demonstrates the conversion of chitin to chitosan under alkaline deacetylation.

Three functional groups are present in the chitosan chemical structure: an amino/acetamido group at the C-2 position, primary hydroxyl group at the C-3 position, and secondary hydroxyl group at the C-6 position, which renders chitosan more chemically active than chitin [4]. The hydroxyl (−OH) and amine (—NH_2) functional groups in chitosan allow various derivatives to be prepared from the original structure, often with properties that can be tailored to suit particular purposes [2]. The amino groups are the deciding factors in the structure and consequently the characteristics of chitosan [8]. Majority of the bioactive properties of chitosan, including its analgesic, hemostatic, antimicrobial, and antioxidant properties are influenced by its physicochemical characteristic; to name a few: molecular weight, degree of deacetylation, and moisture content [4]. Table 2 below demonstrates the physicochemical characteristics affecting chitosan properties.

1.1. Relationship between physicochemical properties and haemostatic ability of chitosan

A comparative study involving solid-state chitosan soliquoid, chitosan acetic acid physiological saline solution, and carboxymethyl chitosan physiological saline solution was carried out to study the effects of chitosan molecular weight and deacetylation degree on hemostasis. The study reported several conclusions. All three types of chitosan revealed different hemostatic mechanisms. Solid-state chitosan is able to absorb platelets to induce coagulation, but lacks the ability to cause erythrocyte aggregation. Chitosan acetic acid physiological saline solution has the ability to cause erythrocyte aggregation and induce deformation in erythrocytes. Carboxymethyl chitosan physiological saline solution is unable to aggregate erythrocytes, but induces local rouleau formation of erythrocytes [69].

Low deacetylation degree of chitosan is defined as 55–70%, which is almost insoluble in water. 70–85% indicates moderate degree of deacetylation, and 95–100% indicates ultrahigh deacetylation degree. Degradation of the chitosan molecule reduces its molecular weight, which increases its solubility in water. Previous comparative studies have reported that less deacetylated chitosan adsorbed more platelets. Meanwhile, hemostasis effective range is with chitosan with molecular weight between 10^5 and 10^6 [71].

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Table 1
Conversion of chitin to chitosan under alkaline deacetylation with modification.

Stage	Procedures	Specific condition(s) or environment(s), reference(s)
Raw material	• The shell wastes are washed, grinded im a blender and sieved into a fine powder (<20 mesh) [71]. • The powder is thoroughly washed with water to remove impurities and dried in a hot-air oven at 90 °C for 6 h [71]. • This material is kept frozen until use [71].	
Demineralization	• Demineralization is done under acid treatment with 7% hydrochloric acid to remove inorganic material such as calcium carbonate [72]. • The reaction is proceeded at room temperature under agitation at 250 rpm for predetermined times (0.5, 2, or 6 h). Afterwards, the demineralized shells are filtrated and washed with distilled water to a neutral pH [71]. • The shells are bleached by immersing in ethanol for 10 min and dried in an oven at 70 °C [71].	• 7% HCl for 24 h [72] • Dilute HCl, room temperature [71], CR [76] • Other acids have also been used (nitric acid, sulphuric acid, acetic acid) [71], CR [76] • The acid concentration and the time of treatment depend on the source of chitin [71], CR [76] • High temperature is undesirable to prevent polymer degradation [71], CR [76] • EDTA prevents degradation [71], CR [73] • Lactic acid fermentation of chitin produces low-quality chitosan as compared to chemical extraction [71], CR [75]
Deproteinization	• Deproteinization involves the extraction of protein matter in alkaline medium (mainly with NaOH), [72] • The process is carried out under agitation at 80 °C for 3 h. The solids are filtrated and washed with distilled water until a neutral pH is achieved. Then, the solids are immersed in ethanol for 10 min for further bleaching, and the resulting chitin is dried in an oven at 70 °C [71] • Prolonged exposure time and high temperature can cause chain scission and partial deacetylation of the polymers [71], CR [76]	• 10% NaOH, 60 °C for 24 h [72] • Dilute NaOH, 65–100 °C from 0.5 to 72 h [71], CR: [76]
Deacetylation (Chitosan)	• The hydrolysis of acetamide bonds results in alkaline deacetylation of chitin that yields chitosan [71], CR [74] • Chitin is reacted with high concentration NaOH [71] • The reaction mixture is cooled down and kept frozen at −83 °C in an ultra-freezer for 24 h [71] • Next, the temperature is increased to 115 °C, and proceeded with agitation at 250 rpm for 4 or 6 h [71] • The resulting chitosan is filtrated, washed with distilled water until neutral pH and dried in an oven at 70 °C [71] • Degree of acetylation (DA) of chitosan, defined as the proportion of acetylglucosamine units in the polymer, will depend on the deacetylation conditions [71], CR [74] • It is challenging to deacetylate chitosan without specific procedures completely [71], CR [74]	• 60% NaOH, 60 °C, for 8 h [72] • Under heterogeneous condition [71], CR [74] • Generally, chitin is reacted with concentrated NaOH or KOH (40–50%) at temperatures above 100 °C [71], CR: [74]

The degree of deacetylation indicates the free amino group content of chitosan. Chitosan with high deacetylation degrees have greater numbers of amino and hydroxyl groups that create stronger hydrogen bonds that are difficult to interact with the blood components, thus lowering hemostatic ability [55]. Hence, solid-state chitosan soliquoid and chitosan acetic acid physiological saline solution are more efficient at hemostasis. Hu stated in chitosan with similar degrees of deacetylation, higher molecular weight chitosan had better coagulation effects; this was supported by reports by Yang et al. [54,67].

2. Engineering and formulations of chitosan products for haemostasis

Chitosan has the exceptional ability to be prepared in different forms, such as sponges, films, granules, powders, lyophilized bandages, hydrogels, beads, microspheres, nanospheres, membranes, fibers, and flakes [2,6]. The simplest method for preparing a hemostatic dressing is by soaking a cotton gauze or bandage in chitosan [9]. Lysozymes hydrolyze chitosan into its glucosamine and N-acetyl glucosamine components; thus, it is biodegradable [10,11]. However, hemostatic chitosan powder is non-bioabsorbable because it forms large aggregates; hence the material needs to be extracted from the wound site before proceeding with surgery to mend the injury [11]. It is very important to abide by the manufacturer's instructions when using chitosan based hemostatic agents.

Various studies and improvements are ongoing to increase the hemostatic ability of chitosan, including combining different materials into hemostatic chitosan formulations. These materials have been proven experimentally to have a synergistic effect with chitosan, which maximizes its hemostatic ability. The most promising form of biomaterial for hemostasis maintenance is hydrogel, as it is not only durable, flexible and absorbent, but also amenable to the addition of various bioactive compounds.

Two types of hydrogels can be produced, depending on the type of crosslinking applied. Hydrogels produced with physical cross-linking have shorter life spans and are reversible, causing them to lose their integrity when exposed to different pH or temperatures. Meanwhile, hydrogels produced via chemical cross-linking are more durable to surrounding conditions but can be toxic from the use of specific chemical agents in its formulation [9,12].

Chitosan hydrogels should have high porosity (90% and above) to be highly absorbent of aqueous solutions and participate in blood clot formation. To assist with proper wound healing, water vapor transmission rate in aerogels before transforming into hydrogels must be high. L-aspartic and L-glutamic acids can be modified as crosslinkers to form the amide bonds. This prevents valuable free amino groups that confer chitosan with its excellent bioactive characteristics from being lost during processing [9].

To enhance the haemostatic effects of chitosan-based haemostatic agents, a higher surface area is desirable to promote interaction between chitosan and the platelets. This can be achieved by micellization and incorporation of the chitosan molecules onto a porous template that acts as delivery vessels.

Table 2
Physicochemical characteristics affecting chitosan properties.

Property	Physiochemical characteristics affecting the property	Remarks	References
Biodegradability	Degree of deacetylation, distribution of acetyl groups, molecular weight	Biodegradation of chitosan by human lysozyme or any enzyme that hydrolyzes glycosidic bonds.	[9]
			[11]
Biocompatibility	Degree of deacetylation	Chitosan is biocompatible with human dermal fibroblast (HDFs)	[11]
Mucoadhesion	Degree of deacetylation, molecular weight		[83]
Hemostatic	Degree of deacetylation, molecular weight	Chitosan nanoparticles	[11,55,67]
	Grades and forms of chitosan derivatives	Water soluble substitution enhances protonation	[62]
	Substituent group	As chitosan is insoluble in water, many properties of chitosan exist only in its acidic solution. Human blood rapidly coagulates in chitosan acid solution.	[54]
	Acid salts	Anticoagulant effect of chitosan is seen in human plasma treated with chitosan sulfate. Chitosan Sulfation is obtained after reaction with chlorosulfonic acid and Dimethylformamide. Sulfated chitosan is the nearest analogue to heparin.	[54,63]
			[65]
		Addition of carboxyl group to chitosan sulfate increases the anticoagulant activity due to higher resemblance to heparin. Carboxymethyl chitosan sulfate showed greater inhibition against the transformation of fibrinogen to fibrin than chitosan sulfate.	[72], (CR: Nishimura, Nish, & Tokura, 1986)
Analgesic	Degree of deacetylation	Presence of positively charged free amino groups coming from aminoglucose mers is also responsible for analgesic effects which occur due to ions being released in the inflammatory area (using chitosan with l-aspartic and l-glutamic crosslinkers)	[9]
		Water soluble chitosan suppresses the expression and secretion of pro-inflammatory cytokines such as tumour necrosis-α, interleukin-6 and induces nitric oxide synthase in astrocytes.	[80], CR: [79]
		The human body synthesizes N-acetylglucosamine as an anti-inflammatory agent. Chito-oligosaccharides, which have a molecular weight of 5 kDa, showed better anti-inflammatory action than the nonsteroidal anti-inflammatory drug, indomethacin.	[80], CR: [78]
		Chitosan inhibits prostaglandin E2 and cyclooxygenase-2 protein expression and attenuates the pro-inflammatory cytokines (e.g., tumour necrosis factor-α and interleukin-1β).	[80] CR: [53]
		Chitosan treatment increases the expression of the anti-inflammatory cytokine, interleukin-10	[80] CR: [53]
Antimicrobial	Molecular weight	Chitosan is antibacterial against *Staphylococcus aureus* and *Escherichia coli.*	[9]
Antioxidant	Degree of deacetylation, molecular weight.	Antioxidant activity due to free amino groups and pyranose rings	[9,11]

Macroporous templates have been used to assemble chitosan in wound healing. The porous template must be made hydrolytically degradable, thus making it biodegradable. A research employed the macrocyclic oligosaccharide, β-cyclodextrin, crosslinked with dianhydride to produce a polyester-linked porous gel, cyclodextrin polyester (CDPE) that is capable of hydrolytic degradation under physiological conditions. This absorbable porous template is able to assemble nanostructured chitosan, thus increasing the surface are and haemostatic ability with significantly lower amounts of blood loss and shorter times to hemostasis [50].

Chitosan microspheres are a potential bone and periodontal filling material and can act as thrombospheres [13]. However, hemolysis can happen as a result of bio-compatibility, attributed to the electrostatic interactions [14,15]. Nevertheless, the potential for hemolysis when using chitosan is less than 10%, indicating its general safe use in hemostatic applications, as well as its amenability for delivery via intravenous means [16]. In further observations, it was shown that chitosan promotes adhesion in red blood cells without damaging the plasma membranes, evident in the high levels of agglutination coupled with low hemolysis rate [13].

Chitosan readily fills in as an alternative for thrombin in platelet-rich plasma (PRP) preparations. The synergistic effect activates and induces platelets to release growth factors. In the study, the chitosan weighed 450 kDa with a deacetylation degree of 490% (Primex Ingredients AS, Avaldenes, Norway) [17]. Chitosan-Gelatin-Ibuprofen films reduce bleeding in surgical operations [18]. Chitosan-Mesoporous Bioactive Glass (MBG) films might be a novel hemostatic agent [19]. The addition of gallium ions in low doses in Chitosan-MBG produces Chitosan-Gallium-MBG, which showed improved aggregation in platelets and blood clotting. Chitosan-Gallium-MBG incorporated with the antibiotic gentamycin showed improved properties in the chitosan scaffolds, including better hemostatic ability, higher antibacterial activity, and improved biocompatibility compared to the commercial Celox^{TM} [8,20].

Sponges made from hydroxyl-butyl chitosan promote vicious agglutination of erythrocytes. By using vacuum freeze-dried techniques, the sponges were more porous (~85% porosity), showed 25-fold better absorbency, improved texture, significant antibacterial action and no toxicity [21]. Fibers are permeable, non-woven dressings. As an example, chitosan coated with polycaprolactone/calcium carbonate nanofibers. The chitosan coating changed the material surface wettability from hydrophobic to hydrophilic, assisting in blood coagulation [8,22,23].

Other forms of chitosan such as chitosan-silica xerogels beads activate the blood coagulation pathway with no reported cytotoxicity [24]. Chitosan-kaolin is synergistic as kaolin can activate Factor XII when exposed to plasma [25]. Carboxymethyl chitosan, sodium alginate and collagen (CSCM) activate platelets and assist in their adhesion due to the numerous surface promontories that increase the total surface area for adhesion of blood cells and platelets. Additionally, it activates the basic coagulation pathway resulting in the development of thrombins [19,26]. Chitosan-Diatom silica stops bleeding via rapid absorption of plasma effusion and erythrocyte aggregation.

Compared to traditional dressing materials and QuikClot® (Z-Medica Corporation), chitosan-diatom induced blood clots studies in a rat-tail amputation model in the fastest time, with minimal loss of blood [27]. Chitosan-polyphosphate and polyphosphate are procoagulants; chitosan-polyphosphate absorbed more blood than chitosan alone to reduce bleeding time. In a *P. aeruginosa*-highly contaminated wound model, the addition of silver to dressings decreased mortality rate from 90% to 14.3%, as compared to standard gauze model [8,28].

A study attempted to produce chitosan dressing with efficient haemostatic and wound-healing properties. The team prepared chitosan nanoparticles using ionic gelation method and assembled the nanoparticles onto a porous chitosan dressing via lyophilization. In the ionic gelation method, positively-charged amino groups of chitosan interact with the negatively-charged crosslinker, sodium tripolyphosphate (STPP), to form strong inter- or intramolecular linkages. Using the thrombin generation assay, chitosan nanoparticles were proven to accelerate thrombin generation. The resulting dressing is highly porous, with enhanced swelling abilities, controlled biodegradation and good biocompatibility. Pore size and surface area are vital parameters of porous wound dressings as they determine fluid absorption rates and healing efficiency of the materials at the wound site. The swelling rate of chitosan must be high enough to absorb high amounts ofexudate and allow better healing. The high hydroxyl and amino group content of chitosan may be responsible for the dressing's high swelling ability [62].

Bulk density reflects the porosity of chitosan. Lower density values correspond to higher porosity and a greater surface area of particles available to interact with blood. Chitosan with lower density values allows the dressing to cover a larger injury area compared to a denser chitosan powder. Bulk density does not correlate with blood clotting activity, as low bulk density chitosan (e.g. 0.33 g/cm3) shows no blood clotting activity, while Celox with a bulk density of 0.56 g/cm3 performs as a highly active hemostatic agent. Lower bulk density chitosan was prepared by precipitation and desiccation. Treatment of chitosan sediments with ethanol or acetone at high concentrations leads to an increase in bulk density and subsequently a decrease in porosity of the gel particles, perhaps due to increased dehydration and tighter packing of the chitosan polysaccharide chains [81].

3. Reputation of chitosan as hemostatic agents

A hemostatic agent is defined as a substance that promotes the stoppage of bleeding. Interestingly, these agents have different surface properties compared to blood-contacting materials that are non-thrombogenic [29]. The red blood cell (RBC) is covered by a fluid lipid bilayer membrane with embedded proteins and glycoproteins. The RBC membrane is negatively charged due to the carboxyl group present on the sialylated glycoproteins embedded in the membrane. The negative charge prevents agglutination between cells, as the electric zeta potential created repulses the cells from each other [30]. Being cationic, the positive charges on chitosan draw in the anionic red blood cells.

Several *in vivo* studies were carried out on animal models such as rabbits, sheep, pigs and dogs to analyze the clotting efficiency of chitosan. Some of the experiments applied fatal experimental designs and allowed uncontrolled prolonged hemorrhage before application of the chitosan dressing. Ozlem introduced severe bleeding in the femoral artery of rats under hypothermia or under oral anticoagulant medications and later applied Celox™ [35]. Celox™ is a hemostatic agent that performs in normal and hypothermic temperatures as well as under anticoagulant therapy [35]. Mirzadehl in his study concluded that treatment with chitosan microcrystalline powder in punctured carotid arteries of sheep significantly reduced manual compression time in the wound site ($p < 0.009$) [36]. In another study, 48 swine were injured in the groin via transection of the femoral vessels. Uncontrolled bleeding was allowed to proceed for 3 min. Later, the animals were randomly divided and assigned to different treatment groups: treatment with normal gauze dressing (SD); treatment with Celox™; treatment with HemCon® dressing (HC); and treatment with Quikclot® dressing (QC). Treatment was applied with 5 min of manual pressure followed by a standard field compression dressing. The subjects treated with chitosan-based dressing had improved hemorrhage control and survival [37].

Millner et al. 2010 experimented on 12 moderately heparinized swine; the animals' femoral arteries were traumatized and allowed to bleed. After treatment with Omni-Stat™, 10 out of 12 achieved hemostasis following the first treatment. The remaining two achieved hemostasis after the second treatment with Omni-Stat™. Fischer reported the macroscopic formation of clots around chitosan fiber hemostatic agents including chitosan-coated sutures, N-acyl-chitosan, tropocollagen chitosan fibers, and N-modified fibers that were introduced in the jugular and femoral vein lumen of a dog model. Another experiment reported a significant reduction in post-treatment blood loss and improved hemostasis in severe hepatic wounds in pigs following treatment with chitosan acetate salt dressing [38].

In severe grade V-classified liver injury, perihepatic packing is done with a gauze pad and compression to stop the bleeding and allow further time for resuscitation and correction of hypothermia, acidosis, and coagulopathy. Chitosan has shown effectiveness in arresting hemorrhage in both normothermic, noncoagulopathic and hypothermic, coagulopathic models for grade V liver injury models which carry high mortality rates. Chitosan demonstrated reduction in post-traumatic bleeding, with low resuscitative fluid requirement and an increased mean arterial pressure during resuscitation [54].

However, chitosan is not always unrivaled. Whilst the HemCon chitosan dressing is a successful hemostatic agent in a normothermic and noncoagulopathic models for grade V liver injury models, the material is ineffective in aortotomy two hours post-surgery. This raises concerns on the safety of chitosan use in high pressure vessels, as there is a risk for re-bleeding [54,56]. Later, Woundstat was reported to be almost 100% effective in in stopping severe artery bleeding when compared to HemCon, Celox and QuikClot ACS+ [58].

Hemostatic control using chitosan bandage has been reported to be less than satisfactory in two cases of extremity injuries [59]. HemCon, a mucoadhesive haemostat agent is not flexible in narrow wounds such as lacerations. However, newer generation materials, such as the flexible Chito-flex have been produced in response to this limitation [58].

Two studies evaluating chitosan bandage use in lethal extremity injuries have reported limited hemostatic control. In addition, a recently developed chitosan patch (ChitoSeal, Abbott Laboratories, Abbott Park, IL) performed worse than standard gauze pads in coagulopathic splenic hemorrhage models [54]. As a result, several improvements have been made to increase the stability and mechanical strength of the formed clot. This includes the development of PolyStat that improves mechanical stabilization of red blood cell aggregates via fibrin cross-linking and anchoring of fibrin to the gauze fiber surface in chitosan dressings [57].

4. Chitosan as dental haemostatic agents

A study was done on 40 patients who underwent dental extractions while maintaining their existing antiplatelet medication prescriptions. In a consecutive follow-up of seven days, patients treated with chitosan-based dressing were reported to have bet-

ter healing and pain control compared to the control [31,32,69]. Moreover, another study also reported significantly less bleeding time in patients on antiplatelet medication when treated with chitosan-based dressing following dental extraction when tested against platelet-rich fibrin [33]. Chitosan remains efficacious in coagulopathic patients as it is safe to be used post-extraction in patients with cirrhosis provided medical management is appropriately addressed [34]. Under single anti-platelet therapy, chitosan is a good alternative [60].

5. Mechanism of chitosan: dental hemostatic agent

Despite its specific pathway remaining undiscovered, hemostasis is theorized as sorption of plasma, hemagglutination, platelet adhesion, aggregation, and activation [9]. Although it is not the primary explanation for hemostasis induced by chitosan, blood sorption is critical because future events and material effectiveness strongly rely on this stage. Chitosan polysaccharides can adsorb between 50–300% of their primary weight in plasma, which consists of erythrocytes, platelets, fibrinogen, and clotting factors in the wound site [39]. Adsorption is directly related to the degree of deacetylation, molecular mass, and the type of chitosan. In chitosan with high degrees of deacetylation, adsorption is higher due to the affinity for water molecules to be absorbed onto the active centers of polysaccharides. Deacetylated chitosan readily binds to fibrinogen, which aids in platelet adhesion [9].

The second mechanism for chitosan hemostasis action is explained by the protonated amine groups that aggregate with the negatively charged red blood cells [39]. In a study comparing high molecular weight chitosan with varying degrees of deacetylation, it was found that moderate degrees of deacetylation had same coagulation times as the positive control. The degree of deacetylation affects the physic-chemical properties of chitosan, as it indicates the amount of free amino groups present in the chitosan structure.

An appropriate quantity of amino groups in chitosan constructs a strong mesh-like entanglement with erythrocytes. In contrast to chitosan with more deacetylation, the presence of more amino and hydroxyl groups formed stronger hydrogen bonds inside the molecule, thus reducing interaction with blood components to promote coagulation [21]. Direct binding of low-molecular weight chitosan to erythrocytes was also predicted as the mechanism of hemagglutination. Most chitosan-based companies such as Celox™ and Hemcon® have stated that the leading role of chitosan is to clot with erythrocytes to stop bleeding.

The leading cause of chitosan's hemostatic effect is related to platelet adhesion, aggregation, and activation. Human platelet activation is determined by aggregation, adhesion, and α-granule membrane glycoprotein expression [17]. Previously, platelets have been shown to bind to chitosan in the presence of plasma proteins [9]. In recent years, ADP and glycoprotein Ib/IIIa receptors have been manipulated for antithrombotic strategies with drug compounds, such as ticlopidine, clopidogrel, abciximab, eptifibatide, and tirofiban to reduce vascular complications.

Previous research has demonstrated that novel oligochitosan can promote the expression of platelet glycoproteins IIb/IIIa and $P2Y_{12}$ to induce platelet aggregation in vitro. Glycoprotein IIb/IIIa (GPIIbIIIa) is an integrin complex and fibrinogen receptor found on platelets that are calcium-dependent and vital for adherence to the endothelium [40]. Some studies suggest that chitosan induces the discharge of thromboxane A2/ADP. The trigger increases platelet spreading and improves adhesion strength [9,19].

Chou et al. observed increased Ca^{2+} ion mobilization and enhanced expression of GPIIb/IIIa complex as part of platelet adhesion and aggregation in a dose-dependent manner. Physiologically, platelets do not adhere to the endothelial wall unless collagen is exposed. Unstimulated platelets maintain very low cytosolicfree Ca^{2+} concentrations and stimulation with platelet agonists such as thrombin and collagen can significantly raise the levels of cytosolic-free Ca^{2+} [70].

Platelet activation under chitosan stimulation was observed using anti-CD61 monoclonal antibody with flow cytometry. A novel study reported the release of EGF, PDGF-AB, and TGF-b1 from platelet-rich plasma (PRP) when chitosan was used as a substitute for thrombin [17]. Another finding suggests that chitosan induces the activation of platelets by analyzing the expression level of P-selectin [41]. The stages of healing following extraction mirrors the classic wound healing pattern: coagulation and hemostasis, inflammation, proliferation, and modeling and remodeling of the alveolar bone.

5.1. Hemostasis and coagulation

Post-tooth extraction, blood immediately accumulates in the wound site from the injured vessel. Activation of platelets and coagulation factors form a thrombus to seal the area, which later acts as a scaffold for cellular interplay during the healing process. As a traumatic procedure, tooth extraction causes microvascular damage and hemorrhage. Early reflex vasoconstriction occurs as a result of retrenchment of the smooth vascular muscle cells, but this process is transient, as bleeding will persist if no fibrin is formed [42].

Chitosan is an important hemostatic option in coagulopathic patients, as it induces coagulation electrostatically, independent of the coagulation cascade. Furthermore, chitosan induces platelet activation [17] and forms a stable clot that seals the area and significantly reduces the bleeding time [33]. However, blood tests such as total blood count, clotting assay, Prothrombin Time(PT), Activated Partial Thromboplastin Time(APTT), and International Normalised Ration (INR) should not be left out during treatment in studies to prevent further complications, as patients with INR of 1.4 and higher were infused with fresh frozen plasma pre-operatively [34].

Sponge, dressing, and granules are among the three common forms of chitosan used to pack post-extraction sockets to achieve hemostasis. Chitosan has no intrinsic hemostatic property. It is considered as being independent of the coagulation cascade and remains efficacious in coagulopathic patients. However, the electrostatic attraction between the red blood cell membranes and chitosan as a result of their opposing charges forms a strong tissue bond that seals the wounded area [36,43,44]. Firm pressure must be applied for several minutes after it is placed in the wound.

Some chitosan dressing can be left in the wound to resorb after several days. In contrast, the other chitosan dressings must be rinsed with saline irrigation from the wound site after hemostasis is achieved. This depends on the manufacturer's instructions. Typically, chitosan is biodegradable and biocompatible. However, careful attention needs to be paid to chitosan powder as it forms large clumps, therefore, care must be taken to extract the chitosan from the application site before proceeding with surgery to mend the injury [11]. This indicates the potential for altering the properties of chitosan during manufacturing. As previously stated, chitosan from different sources and preparation methods carry varying physico-chemical properties that allow different chemical modifications to be made to the structure. Chitosan can be prepared as gels, foams, films, sponges, etc. The number of chitosan-based dressings that can be produced are numerous, each with their own specific physico-chemical and bioactive properties [45].

5.2. Inflammation

The inflammation phase is transitory and involves both humoral and cellular inflammatory responses. Neutrophils phagocytose clots, foreign bodies, and bacteria by releasing enzymes and free

radicals. Later, monocytes are recruited from mature circulating monocytes to continue phagocytosis and release active growth factors that are essential to the activation of fibroblasts and osteoblasts. These factors include: transforming growth factors -alpha and -beta, fibroblast growth factor, and epidermal growth factors. In response to signals from the breakdown of local products and Interleukin-1, lymphocytes are the last recruited members in this phase.

Chitosan studies show poor/absent neutrophil activation as the production of reactive oxygen species (ROS) is lower than the control. The addition of chitosan to polymorphonuclears (PMNs) does not affect the levels of lysozyme released by the cells when compared to PMNs without chitosan. Hence chitosan does not induce ROS production in cells. When stimulated with phorbol 12-myristate 13-acetate (PMA) or formyl-methionyl-leucyl-phenylalanine, PMNs incubated with chitosan showed lower ROS production compared to positive controls cells without chitosan [45]. This makes chitosan suitable for wound healing, as it does not trigger excessive inflammation. The in vitro results indicate that PMN stimulates the granulocyte-colony stimulating factor (G-CSF). Chitosan accumulates osteopontin messenger RNA (mRNA) and releases osteopontin into the culture supernatants [46]. The unique immunostimulatory effect of chitosan is owed to its N-acetylglucosamine, which stimulates chemotaxis and nitric oxide production in macrophages [24].

5.3. Proliferation

This event is initiated by fibroplasia and is recognized by the intense migration and proliferation of fibroblasts. Abundant collagen fibers are secreted, and other extracellular protein matrices are synthesized by the fibroblasts. The abundance of extracellular matrices (ECMs) further supports cellular migration and adhesion. Throughout the first seven days of healing, the wound site is loosely spread with cell-rich granulation tissue with intense inflammatory cell infiltration. Early activation of transforming growth factors-Beta 1 (TGF-β1) and fibroblast growth factors -2 (FGF-2) seem to modulate the activation and proliferation of fibroblastic populations, greatly determining the synthesis and maturation of the extracellular matrix and organization of the granulation tissue.

A high degree of deacetylated chitosan supports the proliferation of fibroblasts in serum [47]. This in vitro study suggests that chitosan may be interacting with growth factors present in the serum and potentiating their effect. Nevertheless, the results are in contrast to experiments with keratinocytes, as their mitogenesis is inhibited. In the few days that follow tooth extraction, blood clots are formed in the wound site, which results in high localization of inflammatory cells in the site. Chitosan and its derivatives accelerate wound healing by enhancing the function of inflammatory cells such as polymorphonuclear leukocytes (PMN), macrophages, fibroblasts and osteoblasts. Chitosan also reportedly improves ability of wounds to withstand outside forces and pressure [24].

5.4. Modeling and remodeling

The dynamic interplay of osteoblasts and osteoclasts results in bone modeling (modification in the bony shape and structure) and remodeling (no modification in bone shape and structure). Mediators such as macrophage colony-stimulating factor (M-CSF), receptor activator of nuclear factor kappa B (RANK), receptor activator of nuclear factor kappa B ligand (RANKL), and osteoprotegerin (OPG) modulate the process. Chitosan not only induces differentiation in osteoprogenitor cells, but also supports bone formation in vitro [24]. Meanwhile, scaffolds made of chitosan and dicarboxylic acids stimulate bone generation in vivo [48]. Table 3 below summarizes some commonly used chitosan-based hemostatic agents in dental settings and commonly available chitosan formats.

6. Other dental applications of chitosan

Chitosan has several distinct properties that have attracted its use in biodentistry. Chitosan has been developed as a composite for oral drug carriers with sustained-release properties and increased bioavailability, and has been used to deliver drugs directly to tissues surrounding the teeth, including antibiotics metronidazole, chlorhexidine, and nystatin (antifungal) [11,31,44,49]. Some investigators have worked on analyzing chitosan membranes coated with bioactive materials including bio ceramic-based agents such as hydroxyapatite and calcium phosphate variants, which include tricalcium phosphate (TCP) α and β as promising templates in guided tissue regeneration (GTR) [50].

Tissue and bone engineering involving the preparation of chitosan scaffolds are in progress, as are chitosan-based membrane barriers in periodontal GTR. The physical and biological properties of chitosan scaffolds can be improved by incorporating the chitosan with different polymers, such as [poly(vinyl alcohol), polycaprolactone, alginate, collagen, silk fibroin], biomaterials (hydroxyapatite, β-tricalcium phosphate, silicone dioxide), or bioactive pharmacological molecules (bone morphogenetic protein 2 (BMP-2), vascular endothelial growth factor (VEGF), and bisphosphonate [51]. A mixture of chitosan-collagen increased the production of blood vessels and fibroblasts during wound healing, and offered the possibility for use as a barrier membrane [52]. In tissue engineering, the pre-

Table 3
Commonly used chitosan-based dressings in after-tooth extraction.

Types	Clinical usage to control bleeding
Hemcon®	• Appropriate amounts of loose dressing must be placed in the extraction socket; too much of it can cause a higher pain score. • Using cotton pliers, the dressing is placed into the apex of the extraction socket without force. The top of the dressing should be flush with the crystal gingiva. • The dressing is gently pressed for two minutes and is most effective in the presence of enough blood to wet the contact surface of the dressing. Proper positioning must be inspected before dismissing the patient. • The dressing usually dissolves within 48 h but may take up to seven days. At seven days, the wound site can be irrigated to ensure removal of any residual material.
Celox™	• Celox™ must be poured, packed and pressed for five minutes. • When in contact with blood, Celox™ swells, gels, and sticks together to produce a gel-like clot, without generating any heat. • The removal of Celox™ is also easy. After removing as much as possible by hand, the remaining residue can be irrigated with water and saline.
Axiostat®	• Apply Axiostat on the entire bleeding site with uniform pressure until it sticks to the surface • Once applied, the material should never be attempted to be lifted or removed, even to assess for hemostasis. • Axiostat® can be removed using saline or water irrigation. It will turn into a gel that can be easily peeled away without causing any trauma to the wound. This is one significant advantage in using Axiostat® as it can be wholly removed from the wound without dislodging the already formed clot. • Chitosan used in Axiostat® does not contain any proteins or allergic components; there have been unknown reported allergies since 2011. • However, since it is non-resorbable, it can be kept on the wound up to 24 h and must be removed from the site before wound closure.

ferred chitosan source for production of scaffolds is from fungal sources due to its superior physico-chemical properties in the final product compared to scaffolds made from chitosan from marine sources [53]. However, this ultimately depends on the source and preparation of the chitosan and the scaffolds itself.

7. Conclusion

Chitosan possesses blood-coagulating abilities owing to its positively-charged nature. Chitosan is a versatile biopolymer that can be manipulated and engineered to produce a high-quality hemostatic biomaterial.

Conflict of interest

The authors declare no conflict of interest.

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