



Chitosan and oral mucosal wound healing in dentistry—Journey from non-patented to patent introspection from 2010–2021

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ARTICLE INFO

Received on: 26/10/2021
Accepted on: 30/01/2022
Available Online: 05/04/2022

Key words:

Chitosan, prospection, patent classification, patent analysis, biopolymer, mucosal wound healing.

ABSTRACT

Wounds are frequently formed when the anatomy and physiology in the normal state of the oral mucosa are disrupted. Its recovery is a multi-staged, complicated, and dynamic biological process. While several biopolymeric derivatives are available for wound healing. Chitosan (CS) is among the well-known, innovative, and eye-catching materials. CS is a polysaccharide biopolymer with a lot of potential for boosting wound healing. Most significantly, CS derivatives have piqued the scientific community's interest as a therapeutic and include them in a variety of formulations to facilitate wound healing. The primary goal of this paper is to offer a scientific as well as technical perspective on CS and its derivatives for oral mucosal wound healing activities between 2010 and 2021. Furthermore, the study focuses on the use of various therapeutic alternatives using CS that is presently being evaluated in clinical trials for wound healing, as well as its integration into polymethyl methacrylate to improve its characteristics for use in dentistry.

INTRODUCTION

Chronic oral wound treatments must adhere well to the wound's surface, as well as eliminate infection and accelerate wound healing. As a result, the therapeutic resources employed for this need to have the right mechanical and swelling properties. Treatments for oral ulcerative lesion are now being researched largely to ease pain, minimize inflammation, enhance healing of ulcers, and extend the time between attacks (Vieira *et al.*, 2012). Anti-inflammatories, growth factors, cytokines, glucocorticoids, and adrenal hormones are among the drugs used to treat oral ulcerative lesions, along with basic oral care (Raber-Durlacher *et al.*, 2013). Muscle action and the production of oral saliva on a continuous basis, on the other hand, may impair the efficacy of therapeutic pharmaceuticals, leading to a low therapeutic

concentration of the medicines. As advancements in mucous membrane adhesives, medication delivery agents, permeability improvers, and other technologies develop, the opportunity to use safe and effective medication delivery to treat local or systemic illnesses has grown. Advancements in oral biomaterials have the ability to enhance the treatment effectiveness of oral ulcerative lesion therapies while also minimizing drug waste (Martin *et al.*, 2015). Wound dressings containing silver nanoparticles, that are natural materials, have been utilized to protect against local wound infections. Silver, on the other hand, is a potent preservative with varied degrees of toxicity that can affect the human body (Karim *et al.*, 2016; Wilkinson *et al.*, 2011). As a result, the creation of a biocompatible medication delivery carrier for ulcerative lesions is required.

Chitosan (CS) is non-teratogenic. It is a N-deacetylated chitin derivative that may be successfully digested into a non-toxic and digestible monosaccharide (Bonferoni *et al.*, 2009; Elsabee *et al.*, 2009). It is the second most prevalent biodegradable polymer in nature, and its antimicrobial, anti-inflammatory, anti-thrombotic, blood clotting, wound-healing, and immunomodulating capabilities

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make it useful in biomedicine (Lewandowska, 2015; Wang and Wang, 2011). CS polymers have lately been used in cell stents and wound healing polymers (Berger *et al.*, 2004; Kalambettu *et al.*, 2012; Nettles *et al.*, 2002). The surface affinity arrangement of the double electron layer generated by CS interactions with mucosal epithelial cells, including its swelling capabilities, assist CS adhesion in the oral environment. It absorbs water, causing the polymer chain to relax from crimped to flat, enhancing structural exposure, and consequently adhesion.

Despite its many benefits, its limited water solubility severely limits its employment in biological applications. The chemical alteration, on the other hand, can boost CS's water solubility and biological activity. Mahattanadul *et al.* (2018) reported that a mouthwash incorporating 0.1% CS turmeric can be utilized as a local therapeutic agent for treating oral ulceration and candidiasis. Zhang *et al.* (2019) developed a Matrine/CS bio-film that had superior antibacterial, biocompatibility, and anti-inflammatory properties during aphtha healing, using biodegradable CS as the carrier material. In their clinical investigation, Shao *et al.*, (2020) discovered that CS can improve drug penetration, attaches strongly to oral mucosal epithelial cells, has a considerable curative impact, and might be used as an ideal medicine in curing oral ulcerative lesions (Zhang *et al.*, 2019).

CS was found to be the most attractive biopolymer for accelerating wound healing since this is not only biocompatible but also is biodegradable and has little to no toxicity (Muzzarelli *et al.*, 1999; Yilmaz, 2004). In addition, CS exhibits antibacterial, mucoadhesive, and hemostatic characteristics (Berger *et al.*, 2004). It is a polycationic polymer having free acetamide as well as hydroxy functional groups attached to the glucopyranose rings that's nucleophile-susceptible (Florea *et al.*, 2006). As a result, CS functionalization could be accomplished by selectively altering free amino groups (Delgadillo-Armendariz *et al.*, 2014; Schuetz *et al.*, 2008). CS is employed for wound healing via formulations like hydrogels (Alves and Mano, 2008; Patois *et al.*, 2009) sponges, films, and porous membranes. CS's physicochemical properties can also be further tweaked to adapt to the wound environment and promote healing (Berger *et al.*, 2004; Thanou *et al.*, 2002). Furthermore, CS derivatives (CD) with increased solubility, antibacterial activity, and the ability to create complexes with medicines or genetic material have been reported (Chen *et al.*, 2006; Janvikul *et al.*, 2006). Therefore, we aim to give a scientific and technological prediction for the use of CD in wound healing

from 2010 to June 2021 through this review. Aside from that, the paper additionally concentrates on toxicity, applicability for usage in dentistry as reinforcement into polymethylmethacrylate.

HISTORICAL PERSPECTIVES OF CS

Henri Braconnot, a French chemist, discovered an insoluble residue after extracting a fungus (*Agaricusvolvaceus*) in 1811, which he termed fongine. Later, French scientist Charles Rouget transformed chitin material soluble in acid by treating it chemically under reflux with potassium hydroxide. Charles Rouget was the first researcher to discover that chitin could be deacetylated and used in a variety of applications (Figs. 1 and 2).

Nonetheless, Felix Hoppe-Seyler, a German biologist and chemist, invented the name "Chitosan" after treating the shells of various species (spiders, scorpions, and crabs) with caustic potash at 180°C and obtaining a substance he named "CS." In acetic acid and dilute hydrochloric acid, this component was soluble. Furthermore, the product had been precipitated by alkali and was degrading at temperatures around 184°C (Crini, 2019). Crini (2019) presented an outstanding study on the history of chitin and CS, in which they divided the process of discovering and developing these biopolymers into five phases, as shown in Figure 3 (Meyer and Pankow, 1935). It begins with the earliest stages of discovery and progresses to modern applications.

In the mid-1930s, X-ray diffraction data (Clark and Smith, 1936; Muzzarelli, 1983) and subsequently enzymatic analysis via infrared spectroscopy was done to deduce the structure of CS as well as chitin (Crini, 2005). It was initially used in the papermaking business in 1936. Rigby was awarded with two patents, first one for the production of CS from chitin and the second one for the development of CS fibres and films.

However, a lack of appropriate manufacturing facilities, as well as fierce competition from synthetic polymers, hampered their commercialization. In the 1970s, "re-discovery" and renewed interest prompted the need to improve the utilization of biowaste from crustaceans of the sea (Berezina, 2016; Khoushab and Yamabhai, 2010; Muzzarelli *et al.*, 2012; New *et al.*, 2011). Several companies began producing and marketing CS as well as chitin products. Since the 1970s, a large number of patents have been submitted, and a large body of scientific literature has accumulated (Annu *et al.*, 2017; Karthik *et al.*, 2017). Chitin, CS, and their many derivatives have over 2,000 applications today (Philibert *et al.*, 2017).

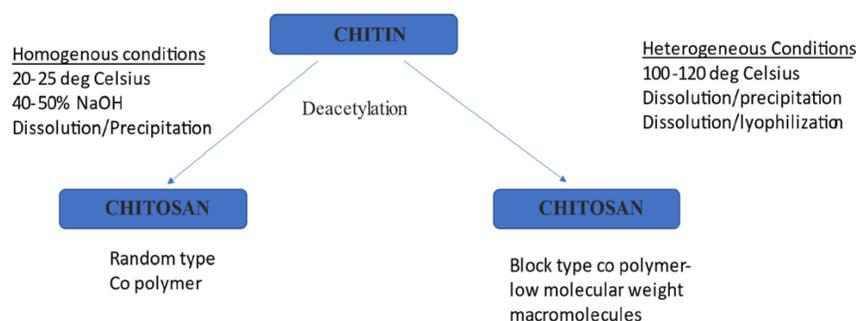


Figure 1. Deacetylation of chitin for CS production.

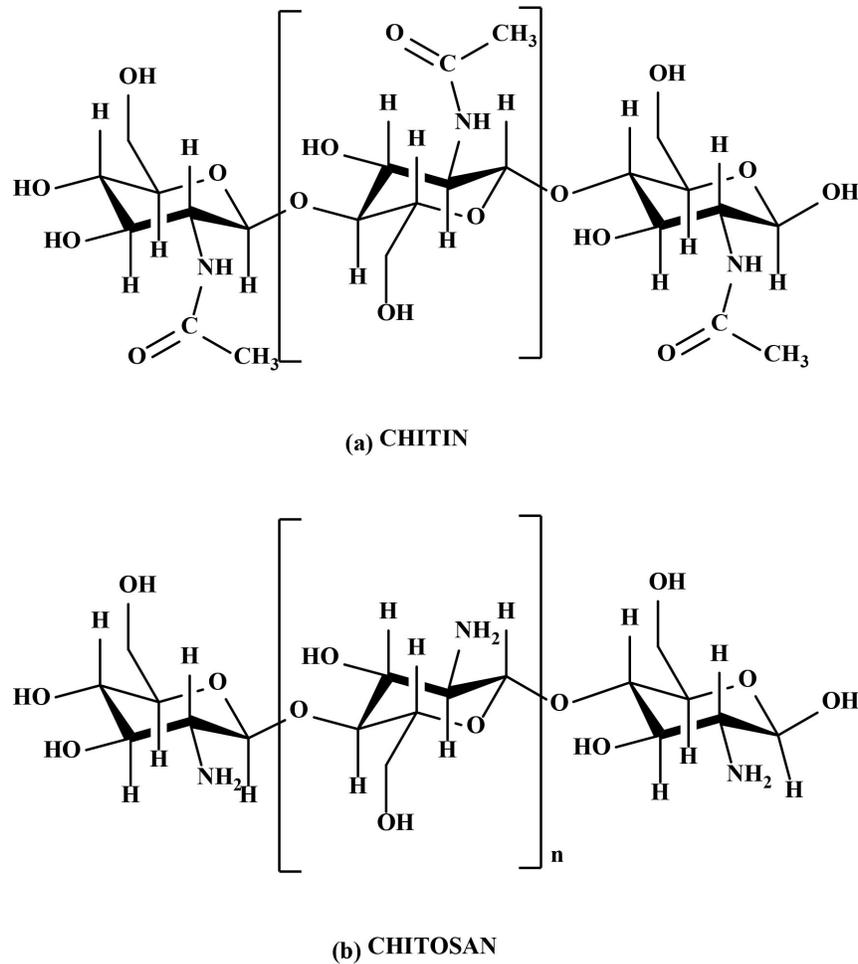


Figure 2. Chemical structure of 100% acetylated chitin (a) and CS (b).



Figure 3. Depiction of the production of chitin, CS, and derivatives from aquatic crustacean biowaste.

Nontoxic, mucoadhesive, hemocompatible, biodegradable, and anti-cancer, antioxidant, and antibacterial characteristics are only a few of the biological features of CS. These characteristics make CS a very appealing biomaterial for a variety of biological applications.

Nontoxicity

CS does not induce severe inflammation nor stimulates the immune system, which is one of its most remarkable qualities.

CS of varied molecular weights and deacetylation levels has been found in studies to have low toxicity, similar to succinyl-derived CS and CS nanoparticles (Aiping *et al.*, 2006; Chien *et al.*, 2016; Huang *et al.*, 2004; Yan *et al.*, 2015).

Antimicrobial activities

CS solutions usually have bactericidal and bacteriological characteristics owing to the polymer's cationic

nature. The polymer chain's positive charge will stick to bacterial surfaces, causing alterations in the membrane wall's permeability, which will limit microbial growth (Goy *et al.*, 2009). CS with a low deacetylation level and a low pH offers better antibacterial properties. Antibacterial activities opposing Gram-negative bacteria can be increased and antibacterial activities opposing Gram-positive bacteria can be decreased by reducing the molecular weight. Furthermore, CS has antibacterial effects against Gram-positive as well as Gram-negative bacteria, with a high mortality rate due to CS and its derivatives interacting with the bacterial cell wall (Younes *et al.*, 2014). The hydrophilicity of the cell wall is required for CS to engage with the bacterial cell, which may explain why CS is less harmful to mammalian cells (Kong *et al.*, 2010).

Mucoadhesive

CS's capacity to bind to surfaces is one of its most crucial features. This characteristic allows for new ways to distribute beneficial chemicals through mucosal channels, as well as the adsorption of molecules that have little affinity for mucus (Bugnicourt and Ladavière, 2016). CS helps to open the tight epithelial junction by increasing the adhesivity of polymers by penetration (Yamamoto *et al.*, 2005).

Hemocompatibility

CS has been utilized extensively in coagulation research. CS can hasten wound healing by interacting with platelets and amino groups on the CS surface (Okamoto *et al.*, 2003). CS's hemostatic qualities have long been utilized in wound healing. CS has chemoattraction, macrophages and neutrophils stimulation, fast granulation tissue formation and re-epithelization, low scar formation and contraction, analgesic qualities, blood coagulation, and intrinsic antimicrobial capabilities as a wound dressing agent (Busilacchi *et al.*, 2013).

Antitumor activity

Using both *in vitro* and *in vivo* models, recent studies have revealed that CS and its derivatives have anticancer properties. The anticancer action of CS is due to an increase in interleukin (IL)-1 and 2 secretion, which causes cytolytic T-lymphocyte maturation and infiltration (Tokoro *et al.*, 1988).

Antioxidant activity

Antioxidants are known for their health-promoting properties. They keep the body's reactive oxygen radical molecules from destroying membrane lipids, proteins, and DNA (Ngo and Kim, 2014). CS and its derivatives have been proven *in vitro* to be able to scavenge active oxygen free radicals. In the removal of free radicals, compared to high-weight CS molecules, low-weight CS molecules provide a number of advantages (Park *et al.*, 2003). On CS, the amino and carboxyl groups stabilize free radicals. This may be the mechanism of CS's antioxidant effect, according to one study (Younes and Rinaudo, 2015).

Biodegradability

Bio enzymes can catalyze the depolymerization of CS in biological organisms. N-acetyl glucose and glucosamine, that are harmless to humans, are the breakdown products. Intermediates of degradation do not pile up inside the body and are not immunogenic.

The molecular weight plus degree of deacetylation of CS have a big impact on its characteristics. The presence of reactive functional groups in CS allows for extensive chemical modification, resulting in a diverse spectrum of derivatives with distinct characteristics. Overall, it's clear that CS and its derivatives are effective carriers for low-molecular-weight medications that need to be delivered precisely.

CS PRODUCTION

CS, a significant chitin derivative, is far more important than chitin. CS, a polysaccharide composed up of D-glucosamine and N-acetyl glucosamine macromolecules, is commercially manufactured after partial deacetylation of chitin (Hudson and Smith, 1998). Although chitin can be found in a range of aquatic creatures, insects, mushrooms, terrestrial crustaceans, and some fungi, due to the availability of large amount of wastes/biowastes from the seafood processing industry, it is primarily commercially collected from marine crustaceans (Hudson and Smith, 1998; New *et al.*, 2014). Shrimp, crabs, lobsters, krill, and squid biowaste are the primary suppliers of chitin and CS on a big scale. Chitin makes up 15%–40% of the marine crustaceans' shells, followed by calcium carbonate (20%–50%) and proteins (20%–40%) (Table 1).

Table 1. CS and chitin sources in terrestrial and aquatic species, as well as microbes (Hudson and Smith, 1998; New *et al.*, 2014).

Aquatic crustaceans	Sea animals	Insects	Mushrooms and fungi	Other microorganisms	Other terrestrial crustaceans
		Scorpions	<i>Agaricus bisporus</i>		
Lobsters	Annelida	Brachiopods	Blastocladiaceae		
Shrimps	Squids	Beetles	<i>Lentinula edodes</i>	Greenalgae	Nematode
Crayfishes	Mollusca	Spiders	<i>Auricularia auriculajudae</i>	Brownalgae	<i>Porcellioscaber</i>
Krill	Coelenterata	Mosquitoes	<i>Trametes versicolor</i>	Yeast	<i>Armadillidium</i>
Crabs	Cuttlefishes	Silkworms	Ascomydes	Spores	<i>Vulgare</i>
		Honeybees	<i>Aspergillusniger</i>		
		Ants	<i>Mucorrouxii</i>		

CS AND ORAL ULCERS

Chronic oral wound therapies must cling to the wound surface while also preventing infection and promoting wound healing. As a result, the therapeutic materials employed must possess the necessary swelling and mechanical attributes. Treatments for ulcerative lesions of the oral cavity are currently being researched primarily to reduce pain, decrease inflammatory response, boost the healing of ulcers, and lengthen the time between episodes (Raber-Durlacher *et al.*, 2013). Anti-inflammatory agents, growth factors, anti-inflammatories, cytokines, adrenal hormones, and glucocorticoids are among the medicines used to treat oral ulcerative lesions, in addition to routine dental hygiene (Raber-Durlacher *et al.*, 2013).

On the other hand, muscle activity and constant saliva production may impair the efficacy of therapeutic drugs, resulting in a low dose of the drug. As technology advances in mucous membrane adhesives, drug delivery carriers, permeability boosters, and other areas, the promise of using effective and safe drug administration to cure local or systemic illnesses has grown. Oral biomaterials research is expanding, with the ability to increase the therapeutic effectiveness of oral ulcerative lesion therapies while decreasing medication waste (Martin *et al.*, 2015). Wound dressings containing natural silver nanoparticles have been used to protect against local wound infections. Silver, on the other hand, is a potent preservative with various toxicity levels in the human body (Karim *et al.*, 2016; Wilkinson *et al.*, 2011). As a result, for ulcerative lesions, a biocompatible medicine delivery vehicle is necessary. CS is an N-deacetylated chitin derivative that may be broken down into non-toxic and absorbable monosaccharides (Bonferoni *et al.*, 2009; Elsabee *et al.*, 2009). It is the second most common biodegradable polymer found in nature, and its antibacterial, anti-thrombotic, anti-inflammatory, blood coagulation, immunomodulating, and wound-healing capabilities make it useful in the field of biomedicine (Lewandowska, 2015; Wang and Wang, 2011). In biomedicine, CS-grafted polymers have recently been employed in cell stents and wound healing polymer dressings (Berger *et al.*, 2004; Kalambettu *et al.*, 2012; Nettles *et al.*, 2002).

CS adhesion in the oral environment is aided by the surface structure of affinity of the double electron layer created by CS interactions with mucosal cells, as well as its swelling capabilities. CS absorbs water, enabling the crimped polymer chain to relax and flatten, boosting structural exposure and stickiness. Despite its numerous advantages, its inability to dissolve in water severely reduces the chances of its utilization in biological applications. However, the chemical modification has the potential to improve CS's water solubility and biological activity. Mahattanadul *et al.* (2018) reported that a mouthwash incorporating 0.1% CS turmeric can be utilized as a local therapeutic agent for treating oral ulceration and candidiasis. Zhang *et al.* (2019) developed a Matrine/CS bio-film that had superior antibacterial, biocompatibility, and anti-inflammatory properties during aphtha healing, using biodegradable CS as the carrier material. In their clinical investigation, Shao *et al.* (2019) discovered that CS can improve drug penetration, attaches strongly to oral mucosal epithelial cells, has a considerable curative impact, and might be used as an ideal medicine in curing oral ulcerative lesions (Zhang *et al.*, 2019).

ORAL MUCOSAL WOUND HEALING

Efficient oral healing process and techniques to aid in uncomplicated oral wound healing are hot areas of interest these days. Inconsistent and prolonged healing times, increased discomfort, allergies, infections, and negative reactions to traditional treatments such as medicated pressure packs under chewing pressure have all been reported.

The healing process is a multi-phased process characterized by redness, swelling, discomfort, and loss of function (Perchyonok *et al.*, 2014).

Wound healing begins with hemostasis, in which the clotting system is activated and platelets contact collagen, resulting in aggregation and activation. To establish a stable clot, fibrin mesh production is initiated. Inflammatory reactions are triggered by this hemostasis. The proliferative phase follows, which includes the development of new connective tissue, granulation tissue, and reepithelialization before the maturation stage concludes the process (Barrientos *et al.*, 2008; Flanagan, 2000). Biomaterials like CS, cellulose, heparin, collagen, hyaluronic acid, and others have been investigated for wound healing in recent years. This function aims to promote wound healing by optimizing regeneration, protecting against infection, ensuring uniform cell distribution, maintaining cell viability and phenotype, and inducing epithelial cell, endothelial cell, and fibroblast migration and proliferation while meeting structural and biocompatibility requirements (Dreifke *et al.*, 2015).

CS, a naturally generated polymer derived from the deacetylation of chitin, possesses properties that make it suitable for usage in a variety of biomedical fields, including tissue healing and regeneration (Huaixan *et al.*, 2016). Its biocompatibility, biodegradability, hemostatic, antibacterial, and wound-healing properties suggest that it could be employed in periodontal therapy, such as oral mucosa wound healing (Silva *et al.*, 2013). CS could be used in hydrogel forms since it is soluble in acidic environments and has a high tissue affinity (Chang *et al.*, 2014; Wongpanit *et al.*, 2005).

Because chitin and CS have antibacterial qualities, they've been employed as wound dressings in the veterinary industry to speed up the healing process. Furthermore, CS oligomers and chitin oligomers, which are produced by an enzymatic breakdown in a wound setting, stimulate macrophages (Chang *et al.*, 2014). CS also is compatible with gingival fibroblasts. It has a synergistic effect with some growth factors, including Platelet-derived-growth factor (PDGF), one of the growth factors that regulates cell development and division as well as angiogenesis and may stimulate cell proliferation in gingival fibroblasts (Silva *et al.*, 2013). Hemon Dental Dressing is a novel CS-based hemostatic oral wound dressing evolved from the US military's HemCon Bandage combat wound dressing. It is made from insoluble shrimp shell chitin that's been thoroughly refined and purified (Gupta *et al.*, 2019).

CS PREPARATIONS BASED ON HYDROGELS

Hydrogels comprise macromolecular networks that expand when water or other biological fluids are present (Nikolaos, 1986). They are good for wound protection because they are easy to use, provide protection, and let air and water pass through. When CS solutions are administered to a wound site, they tend to

gel. Activating *in situ* CS gel formation, according to Berger *et al.* (2004), involves a detailed investigation of pH, temperature, and ionic charge. CS hydrogels were classed as chemical or physical hydrogels by researchers. Chemical hydrogels are made up of irreversible covalent bonds, whereas physical hydrogels are made up of reversible ionic bonds or polyelectrolytes. They are good for wound protection because they are easy to use, provide protection, and let air and water pass through. When CS solutions are administered to a wound site, they tend to gel. Physical and chemical varieties of CS hydrogels were classified by these researchers. Chemical hydrogels are made up of irreversible covalent bonds, whereas physical hydrogels are composed of reversible ionic bonds, polyelectrolyte complexes, secondary couplings between CS and polymeric materials, or grafted CS (Berger *et al.*, 2004). Chemical hydrogels with covalent connections tend to produce stronger gels, which mix with active ingredients and become hazardous. Physical hydrogels, on the other hand, are not limited in this way. As a result, they are regarded as perfect candidates and are receiving increased attention. A limited fraction of CS hydrogels also prefer to gel at the specific tissue or body cavity due to polymer interactions, a process known as *in situ* systems. The formulations of this kind are appealing because they gel when exposed to a body temperature similar to that of a trigger. In response to the stimulus, the formulation changes from an injected free-flowing fluid formulation to a gel formulation. Hence, also known as thermosetting formulations (Patois *et al.*, 2009).

Many different growth factors (proteins) can improve wound healing using CS-based hydrogel compositions. Many growth factors are used in wound healing, and some of the most well-known are transforming growth factor-beta, vascular endothelial growth factor, epidermal growth factor (EGF), and platelet-derived growth factor (PDGF). Alemdaroğlu *et al.* (2006) used a CS topical gel to give EGF to rats with burns. When compared to CS formulations without EGF, which showed partial healing of wounds, EGF-containing formulations dramatically speed up the healing process due to the development of granulation tissue through re-epithelialization.

OTHER FORMULATIONS OF CS

CS has the advantage of being a powder, film, and sponge on the surface of wounds. When secretions from the wound are absorbed, the applied formulation becomes hydrated and transforms into a gel. For example, Mizuno *et al.* (2003) described CS film compositions with basic fibroblast growth factor for wound healing in diabetic mice. The researchers determined that there was an increase in angiogenesis and granulation tissue formation. Noel *et al.* (2010) described CS (porous) sponges for antibiotic administration, specifically amikacin and vancomycin. CS was found to be a viable carrier for local antibiotic medication

administration with the ability to effectively suppress bacterial growth. Jin *et al.* (2007) evaluated the effects of CS powder and together with heparin. The results of the histology investigation after 72 hours demonstrated that CS powder assisted in reducing early burn expansion. When CS was coupled with heparin, however, the benefit was diminished because it was less effective in preventing early burn extension. Nonetheless, Kratz *et al.* (1997) discovered in a similar study, the CS-heparin complex demonstrated healing and full re-epithelialization. It's worth noting that this favorable effect was noticed when heparin was used. It is worth mentioning that this beneficial impact was discovered when heparin was combined (ionic) with CS at a concentration of around 7.7%. As a result, it's reasonable to conclude that to achieve desired therapeutic efficacy, a suitable amount of heparin must be supplied in a regulated manner, which can be accomplished through the formation of an ionic complex with CS.

CS TOXICITY

CS's *in vitro* toxicity is determined by its degree of deacetylation and molecular weight (Schipper *et al.*, 1997). *In vivo* toxicity of CS is dependent on dose and method of administration. Using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) test, Wimardhani *et al.* (2012) studied the impacts of low molecular weight (50–190 kDa) as well as high molecular weight (310–375 kDa) CS microparticles upon keratinocyte cell lines (HaCaT). Comparing low and high molecular weight CS microparticles, there was a two-times increase in proliferation. The IC₅₀ value for low molecular weight CS was discovered to be 800 g/ml, which reduced cell viability by 50% (Wimardhani *et al.*, 2012). Carreño-Gómez and Duncan (1997) conducted a thorough analysis to predict the *in vitro* toxicity of soluble CS (polymers, salt formulations, derivatives such as CS glycol, and CS microspheres cross-linked with glutaraldehyde). For this study, MTT was performed on murine melanoma cell lines (B16F10). According to the results, the CS hydrolysate salt was the least damaging, while the hydrochloride salt was perilous, with an IC₅₀ of 0.21±0.4 mg/ml. Overall, CS hydrochloride was found to be less harmful than CS hydroglutamate, CS Glycol, and CS hydro lactate in tests with cancer cells (Carreño-Gómez and Duncan, 1997). CS does not irritate the skin or eyes and has no pyrogenic impact when applied topically. These characteristics were studied using rabbits and guinea pigs, according to Rao and Sharma (1997).

As previously mentioned, CS toxicity appears to be route-dependent when it comes to *in vivo* toxicity. In mice, Arai (1968) showed that the LD₅₀ value exceeded 16 g/kg when given orally, well above the deadly dosage of sucrose. Additionally, when given orally to rats, Costa *et al.* (2014) discovered an LD₅₀ of over 1,500 mg/kg. Furthermore, when rats and mice were given

Table 2. CTs on the safety and efficacy of CS and also its formulations are included below (in process, recruiting subjects, and completed) till 16th June 2021 (Patil *et al.*, 2021a).

CTs no.	Collaborator	Specific condition	Medicament	CT -phase	NCT identifier
1	Chulalongkorn University and Police General Hospital	Pressure ulcer	CS in combination with sericin (cream)	Completed	NCT04559165
2	University of Brasilia and others	COVID-19	CS nanoparticles	Completed	NCT04490200
3	University of Oslo	Periimplantitis, Peri-implant mucositis	CS brush	Completed	NCT03421717

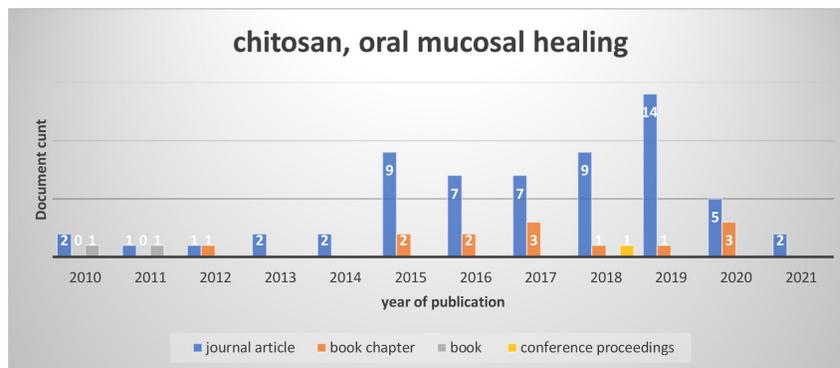


Figure 4. From 2010 to June 2021, the number of publications about CD and CD + wound healing.

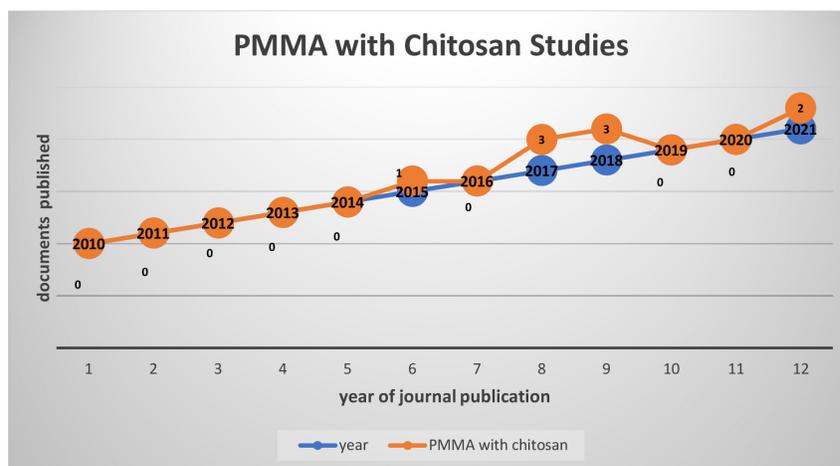


Figure 5. Total number of publications on CS incorporation into PMMA during the period 2010 to 2021.

the drug intraperitoneally, the LD50 values were greater than 3,000 and 5,200 mg/kg, respectively. Based on the findings of this research, it can be concluded that CS has no acute toxicity (Costa *et al.*, 2014). In terms of chronic toxicity, rabbits were administered 4.5 mg/kg/day of CS intravenously for 11 days and no abnormalities were found. A dosage of 50 mg/kg/day, on the other hand, resulted in death three days later. Nonetheless, when CS was administered orally to rabbits at a dosage of 700–800 mg/kg/day for 34 weeks, the toxicity was minimal (Ueno *et al.*, 2001). Based on these data, it is possible to conclude that CS has minimal acute and chronic toxicity. Furthermore, with subcutaneous administration at a high dose of 200 mg/kg, CS induces respiratory distress in dogs, ultimately leading to death. This was most likely owing to increased fibroblast IL-8 production, which culminated in angiogenesis as well as neutrophil migration and deposition in the lungs (Baldrick, 2010). Based on the preceding, it is possible to infer that CS is safe at acceptable quantities but hazardous at excessive dosages. Nonetheless, CS is a safe material to use when used in reasonable amounts. Nonetheless, CS is a safe material to use when used in reasonable amounts. As a result, it is a Generally Recognized as Safe excipient for a broad range of food and therapeutical uses, including parenteral formulations (Ylitalo *et al.*, 2002). Wound healing is just one application for CS formulations. CS's toxicity has been thoroughly investigated using a variety of *in vitro* and *in vivo* testing methods. We could

not find much information about CS's toxicity in people. In one of the human trial assessments, CS was determined to have no major negative effects or allergies. A small percentage of trial participants complained of mild nausea and constipation (Ylitalo *et al.*, 2002). Furthermore, when consumed at a daily dose of 4.5 g, CS was found to be safe in another study (Gades and Stern, 2003).

CLINICAL TRIALS (CTS) AND OTHER SCIENTIFIC DATA ON CS

The CTs on CS conducted to date are shown in Table 2 (Patil *et al.*, 2021a). Also, we conducted a scientific prospection from 2010 to 2021, using LENS as a search database. The phrases “wound healing” as well as “CS derivatives” were searched in both the title and abstract search boxes. The acquired findings are shown in Figure 4.

The following sub-sections describe important literature on CTs of CS for wound healing that is currently available in the public domain.

Pressure ulcers are being studied using CS in conjunction with sericin (NCT04559165). CTs to evaluate the effectiveness of respirators containing CS nanoparticles in the filtration component to prevent COVID-19 have recently begun (1st March 2021) (NCT04490200).

A randomized controlled study was done to investigate the effect of two maintenance programs utilizing titanium curettes

Table 3. List of scientific publications on the incorporation of CS into PMMA as of 16th June 2021 (Chander and Venkatraman, 2022; De Mori *et al.*, 2019; Patil *et al.*, 2021b; Perchyonok *et al.*, 2014; Pushpa *et al.*, 2021; Srimaneepong *et al.*, 2021; Walczak *et al.*, 2018; Więckiewicz *et al.*, 2017).

Author, year	Type of study	Objective of research	CS formulation	Outcome
Walczak <i>et al.</i> , 2018	In vitro	The effect of chemical disinfectants on CS-coated denture surfaces was investigated in this study.	2% CS acetate solution as a coating material	After disinfection on PMMA and Polyethylene terephthalate glycol (PETG), the CS coating was most stable.
Więckiewicz <i>et al.</i> , 2017	In vitro	The thickness, homogeneity and adhesive strength of CS coatings on PMMA denture bases were tested.	4% CS acetate solution	The coating process yielded CS layers with varying thicknesses and overall good adhesion strength. CS Coatings resulted in relatively homogeneous CS thickness, suggesting that they could be used as an intraoral wound dressing
Walczak <i>et al.</i> , 2018	In vitro	This chapter's goal was to present current MMA processing trends.		It covers the chemical production, traditional thermal processing of this acrylic resin, the innovative ultrasound-assisted processing approach, the antibacterial effect of nanoparticles on PMMA, and the material's cytotoxicity, genotoxicity, and mutagenesis.
De Mori <i>et al.</i> , 2019	In vitro	The study found that integrating CS to reduce porosity, silver nanowires for antibacterial effect, and methacryloyl CS (CSMCC) to boost cross-linking with MMA and minimize the quantity of monomer required for polymerization improved the mechanical performance of PMMA-based bone cement.	CS/CSMCC at various concentrations (0%, 10%, 20%, and 30% w/w) or silver nano wires- (0% and 1% w/w)	Cement containing more than 10% CS had a significantly lower polymerization temperature. With a P/I concentration of 2:1, concentrations more than 20% affected mechanical performance. Antimicrobial activity of silver nanowires (AgNWs) against <i>Staphylococcus aureus</i> was seen at concentrations as low as 1% w/w, however, biofilm formation on the cement surface was increased when CS was added to the mix. The combination of AgNWs and CS is permissible for the gradual release of a higher concentration of Ag ⁺ , resulting in increased antimicrobial activity.
Srimaneepong <i>et al.</i> , 2021	In vitro	The study aimed to assess the effectiveness of low-molecular-weight CS solutions against <i>Candida albicans</i> biofilm on PMMA resin. By broth dilution, various types of CS were tested for anti- <i>Candida</i> activity.	30 kDa CS solution and 3 and 6 mg/ml of oligomer CS and	Both types of CS solutions were effective antifungal denture cleaners, significantly reducing <i>C. albicans</i> viability in biofilm on PMMA.
Perchyonok <i>et al.</i> , 2014	In vitro	The current study aimed to assess the efficacy of PMMA containing biomaterials-nano-diamond: CS in the cure and hindrance of denture stomatitis and related circumstances in denture wearers.	By dissolving the appropriate component in glycerol and acetic acid and adding a CS gelling agent, the bio-active nano-diamond modified PMMA was generated.	<i>In vitro</i> bioactive release and potential antimicrobial properties of coated PMMA were demonstrated.
Walczak <i>et al.</i> , 2018	Book chapter	Highlights on the enhancement of mechanical properties of PMMA on the incorporation of CS	CS and poly(acrylic acid) hydrogels with an interpenetrating polymer network.	Could exhibit superior mechanical properties
Walczak <i>et al.</i> , 2018	Book chapter	Highlights on the improvement of ceramic by forming a composite material with CS and gelatin to mitigate the brittle nature of ceramic material	CS (20%) and hydroxyapatite (30%) are used.	Applications of chitosan/h-Hyaluronic acid (HA) composites are utilized in a wide range of scaffold materials in bone tissue engineering and will aid in the guided redevelopment of new bone.

Continued

Author, year	Type of study	Objective of research	CS formulation	Outcome
Walczak <i>et al.</i> , 2018	In vitro	The study concentrated on a local Icarin (ICA) transport system of a phase-transited lysozyme (PTL)-primed Ti surface by a layer-by-layer (LbL) self-assembly system.	The pristine Ti was first covered with a PTL nanofilm. The ICA-loaded hyaluronic acid/CS (HA/CS) multilayer was then applied to form the HA/CS-ICA surface using the LbL system. The established HA/CS-ICA surface was characterized using Scanning Electron Microscopy (SEM), X-ray photoelectron spectroscopy, and contact angle measurement. The ICA release pattern of the HA/CS-ICA surface was also examined.	An ICA-immobilized HA/CS multilayer on a PTL-primed Ti surface displayed a prolonged ICA release pattern, which might stimulate osteoblast osteogenesis <i>in vitro</i> and improve early osseointegration <i>in vivo</i> , according to the current work. This research outlines a novel strategy for building a long-term ICA delivery system that boosts osteoblast responsiveness and osseointegration.
Chander and Venkatraman, 2022	In vitro	In heat polymerized Denture base resin, the researchers investigated at how varying Ch concentrations affected fracture toughness (FT), flexural strength (FS), surface roughness (Ra), and impact strength (IS),	5%–15% if CS incorporated into PMMA was evaluated for FS, FT, IS and Ra.	There was an improvement in FS, FT, and IS CS incorporation. The addition of Ch to denture base resin (DBR) improved the incorporation of “Ra at 15% wt and FT, FS, IS at 5% wt.”

Table 4. List of patent publications on CS and oral wound healing and PMMA incorporated with CS (77–88).

Sl. no.	Country code	Granted patent no.	Title of the invention	Name of the patent applicant	Year published	Purpose
1	US	US 2021/012127 2A1	Method for treating Denture Stomatitis using Denture base material containing Nanodiamonds	Univ. Imam Abdulrahman Bin Faisal	April 29, 2021	Many researchers are actively engaged in not only discovering novel derivatives but also in obtaining patent protection for their ideas, according to the patent study. Overall, our patent analysis will assist many researchers and companies involved in CS research and development in finding a variety of research options and areas where CS, its derivatives, and formulations can be successfully used.
2	US	US 2014/0072657 A1	Novel Pharmaceutical Formulation	Vockner Helmut	March 13, 2014	To treat mucosal and epithelial wounds as well as oral, mucosal, and cutaneous infections and inflammations, the current invention uses innovative formulations including CS and vitamin D or chlorhexidine and vitamin D (or both).

Continued

Sl. no.	Country code	Granted patent no.	Title of the invention	Name of the patent applicant	Year published	Purpose
3	WO	WO 2018/130951 A1	Formulations incorporating CS, as well as methods for making and using them	MarteinssonRunar, Lauzon Helene L, VigfusdottirSigridurVigdis	July 19, 2018	CS-containing formulations, methods of creating such formulations, and methods of using such formulations are described herein to maintain a good condition where they are applied or contributing to health enhancement, healing, illness prevention, and treatment. The current invention additionally includes concentrated solutions that can be used to make other items. The focus of the current invention is tissue regeneration. New production machinery and techniques, new A-PRP or platelet rich plasma (PRP), hyaluronic acid and/or CS compositions, employed alone or in combination, and related devices are also covered. The subject of the current invention is tissue regeneration.
4	WO	WO 2013/061309 A2	New platelet rich plasma (A-PRP) Medical Device & Tissue Engineering Composition, Manufacturing Machines, And Process	Turzi Mr. Antoine	May 2, 2013	The current invention relates to electrospon fibers containing a hydrophilic polymer that is easily resolvable in a first solvent, a bioadhesive constituent such as CS that is relatively solvable in said first solvent, and a medicament material to assist in the recovery of oral wounds
5	US	US10052291 B2	In compositions, electrohydrodynamically produced fibers for the delivery of particular dosages of an active chemical to the skin or mucosa are included.	Hansen Jens, Christiansen Lars Hellaerung	August 21, 2018	CS-glucan derivatives are presented, in which CS is coupled to polylactic acid or an aminoalkyl (aminoethyl) to provide good wound adhesive characteristics.
6	US	US 8835528 B2	Adhesive Composition	Pravata Laurent; and SynolynePharma	April 15, 2012	CS-glucan complexes have been discovered to aid in the healing of the wound and avoid bandages adhering to the wound.
7	EP	EP 2203175 B1	A CS-glucan-based formulation for wound curing and avoiding bandage adhesion	Contipro Biotech Sro		Covalently connecting CS and hyaluronan for wound healing and delivering various therapeutic ingredients that aid in wound healing is disclosed.
8	EP	EP 1753787 B1	Hyaluronan and CS Covalent Linking Method	Wang Wei	October 19, 2019	

Continued

Sl. no.	Country code	Granted patent no.	Title of the invention	Name of the patent applicant	Year published	Purpose
9	CN	CN 102492182 B	Biofilms with CS and/or CD containing rare-earth elements	Sang Q, Chen X, Xiao Q	December 18, 2013	CD either alone or in conjunction with rare earth elements are disclosed, that contain CS. Lanthanum, Samarium, and Cerium are examples of rare earth elements. The mixtures are designed to act as sponges for wound healing.
10	CN	CN 102327284 B	Silver-incorporated CS Nanocomposite Liquid-phase by Synthesis Method	Liaocheng University	October 7, 2012	It also reveals CS nanoparticles with silver nanoparticles for wound healing.
11	CN	CN 107744524 B	An antibacterial gel containing iodine tincture and method of preparation	Qingdao Health Marine Biopharmaceutical Co., Ltd.	August 25, 2020	Describes chitin/CD For wound healing are revealed.

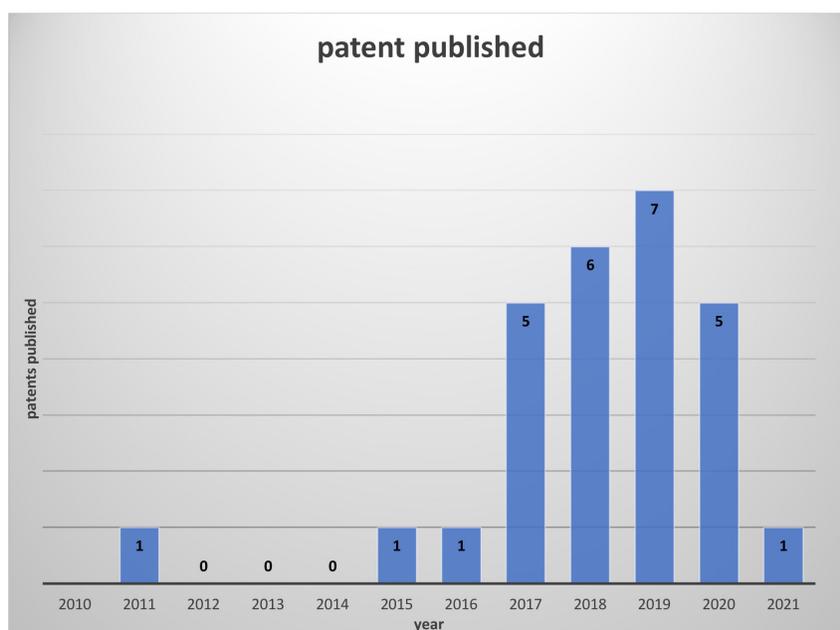


Figure 6. Total number of publications on CS incorporation into PMMA during the period 2010 to 2021.

or CS brushes every third month from 6 to 18 months after surgical treatment of peri-implantitis (NCT03421717). According to the findings, there was no statistically significant difference in treatment procedures requiring more effective submucosal cleaning after peri-implant surgery using titanium curettes or CS brushes.

A total of 93 scientific reports were observed on CD for wound remedial. According to the graph (Fig. 5), the number of publications has increased since 2010, with the highest seen in 2020, with 80 publications focusing on CS, oral wound healing, and 12 publications for CS incorporation into polymethyl methacrylate (PMMA) to improve properties in dentistry.

These data strongly suggest that creating innovative CD for wound healing applications is becoming more popular. In 2011–2012, the number of publications for CD fell, with only two being published. In the year 2017–2018, there were only

seven publications on CD for wound healing. When we searched for publications for the year 2019, we discovered 14 on CS for wound healing in dentistry. In 2021, there was growing interest in incorporating PMMA material in dentistry, with publications focusing not only on wound healing alone but also on a combination of wound healing and antimicrobial effect (Table 3). Out of 80 published articles, 10 articles focused on the usage of CS as a novel ingredient to improve the properties of PMMA and for oral mucosal wound healing.

PATENT PUBLICATIONS ON CS

Following our CS scientific prospection, we concentrated on patent literature utilizing the free internet search resource “Lens.” We began our search by typing “CS derivatives” into the title/abstract/claims search forms. Furthermore, in the same search

boxes, by utilizing a combination of keywords “CS derivatives” and “wound healing.” The literature was searched from January 1st, 2010 to June 16th, 2021. On CS, oral wounds, dentistry, there were 225 patent results as of June 2021, 11 patents have been published on CS and CD, and oral wound curative and incorporation of CS into PMMA (Table 4; Fig. 6). These figures demonstrate that there is a lot of interest in the field of wound healing using CD. The graph shows that patent results reported were highest in 2019. Though there was no substantial increase in CS derivative patents until 2016, following which it shot up to five patents in 2017 and six patents in 2018. There will be five published patents by the end of 2020.

CONCLUSION

CS is a valuable biopolymer for the treatment of wounds. It is a polymer that is non-toxic, biocompatible, and biodegradable. Various *in vitro* and *in vivo* studies are conducted to better understand CS's behavior and any toxicity it may have. Significant research is being conducted on CD in an addition to CS that can cure wounds, according to both scientific and technical research conducted. Our patent investigation will aid CS researchers and companies in identifying different research alternatives and sectors where it is possible to successfully use CS, its derivatives, and formulations used to address oral mucosal healing in general. CD are highly effective at speeding up wound healing ability and improving wound appearance. A study *in vitro* found that the addition of CS to PMMA helped prevent denture-related stomatitis while simultaneously increasing surface Ra. Even though CS showed the anti-microbial property to improve oral mucosal healing, its inclusion into denture material must be thoroughly investigated via CTs. However, more research on characterization is required to eliminate the harmful impacts of CD obtained from starting materials or other sources.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

FUNDING

There is no funding to report.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

Not Applicable.

PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

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How to cite this article:

Meenakshi S, Raghunath N, Ramu R. Chitosan and oral mucosal wound healing in dentistry—Journey from non-patented to patent introspection from 2010–2021. *J Appl Pharm Sci*, 2022; 12(04):012–025.