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"CHITO-OLIGOSACCHARIDE: A MULTIFUNCTIONAL BIOPOLYMER ADVANCING MODERN WOUND HEALING"

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Abstract

Chito-oligosaccharide (COS), a low–molecular weight bioactive derivative of chitosan, has emerged as a highly promising multifunctional biopolymer for advanced wound management. Its unique structural characteristics—short β-(1→4)-linked chains of D-glucosamine and Nacetyl-D-glucosamine with tunable degrees of polymerization and acetylation—provide enhanced solubility, stronger biological interactions, and improved therapeutic efficacy compared with native chitosan. These attributes enable COS to modulate multiple cellular and molecular pathways involved in wound repair, making it a superior candidate for modern wound-care formulations.This review provides an updated and comprehensive assessment of the chemistry, physicochemical properties, and mechanistic actions of COS across all woundhealing phases. COS exhibits potent antimicrobial effects through membrane disruption, biofilm inhibition, and metal-ion chelation, while simultaneously regulating inflammatory responses by suppressing TNF-α, IL-1β, and IL-6 and promoting IL-10–mediated M2 macrophage polarization. It enhances fibroblast proliferation, keratinocyte migration, extracellular matrix reorganization, and collagen synthesis via activation of ERK, PI3K/Akt, and FAK signaling pathways. Additionally, COS supports angiogenesis through upregulation of VEGF, FGF-2, and PDGF, and displays significant antioxidant capacity that protects tissues from oxidative stress.Emerging applications of COS in hydrogels, nanocomposites, scaffolds, membranes, and spray systems demonstrate its versatility in developing next-generation wound therapeutics. Furthermore, synergistic interactions with ions, growth factors, and antimicrobial agents enhance its healing potential. Collectively, COS represents a scientifically validated, safe, and adaptable biomaterial with substantial promise for transforming modern wound-care strategies.

Keywords *– Chito-oligosaccharide (COS); Wound healing; Anti-inflammatory activity; Antimicrobial action; ERK/PI3K-Akt/FAK signaling; Angiogenesis; Biomaterial applications; Antioxidant activity.*

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INTRODUCTION:

Chronic wounds represent a major and growing global health burden, affecting a significant portion of the population and imposing substantial economic costs on healthcare systems. The prevalence of non-healing wounds—such as diabetic foot ulcers, pressure ulcers, and venous leg ulcers—is increasing due to aging populations, rising incidence of diabetes, and vascular disorders [1, 2],. In many countries, wound-care expenditure constitutes a large proportion of health care budgets, and morbidity associated with chronic wounds dramatically reduces quality of life [3, 4].Current wound dressings include gauze, hydrocolloids, alginates, films, foams, and advanced biomaterial-based dressings. However, many of these solutions remain passive and fail to fully address the multifactorial pathophysiology of chronic wounds. These limitations include inadequate antimicrobial coverage, insufficient modulation of inflammation, poor promotion of angiogenesis, and lack of sustained tissue regeneration [5, 6]. Additionally, conventional dressings may not efficiently manage wound exudate, and some require frequent changes, thus raising the risk of infection and increasing treatment cost [7].

Chitosan, a naturally derived polysaccharide obtained by deacetylation of chitin, has been studied extensively in wound healing because of its biocompatibility, biodegradability, hemostatic capacity, and intrinsic antimicrobial properties [8, 9]. However, chitosan's clinical translation is hampered by poor solubility at physiological pH, high molecular weight, and limited diffusivity [10]. These drawbacks constrain its interactions with cells and tissues, and its capacity to actively modulate the wound microenvironment [11].Chito-oligosaccharide (COS), a low–molecular-weight derivative of chitosan, overcomes many of these limitations, making it a superior candidate for advanced wound therapy. COS retains the favorable features of chitosan—such as biodegradability, low toxicity, and positive charge—but exhibits markedly improved solubility and bioavailability [12]. The reduced molecular size and increased functional group accessibility of COS facilitate more efficient interactions with key biological targets, including microbial membranes, extracellular matrix (ECM) proteins, growth factors, and immune cells [13].

Mechanistically, COS is emerging as a "game-changer" in wound care due to its multimodal actions. It exerts potent antimicrobial effects by disrupting bacterial membranes and inhibiting biofilm formation [14], modulates inflammation by downregulating pro-inflammatory cytokines and promoting anti-inflammatory phenotypes in macrophages [15], and enhances tissue regeneration by stimulating fibroblast proliferation, keratinocyte migration, and collagen deposition via signaling pathways such as PI3K/Akt and ERK [16]. Moreover, COS supports angiogenesis through upregulation of vascular endothelial growth factor (VEGF) and other angiogenic mediators [17], while its antioxidant and metal-chelating properties help to mitigate oxidative stress within the wound bed [18].

The combination of these biological effects positions COS as a highly promising biomaterial, capable of addressing multiple barriers to healing in chronic wounds. Moreover, its versatility allows formulation into hydrogels, nanofibers, films, composite dressings, and injectable matrices [19]. Regulatory and manufacturing challenges remain, but the growing body of preclinical and translational data supports COS as a potential paradigm shift in wound therapy [20].

Detailed Chemistry & Structure of Chito-Oligosaccharides (COS)

Chito-oligosaccharides (COS) are oligomeric fragments derived from the partial depolymerization of chitin or chitosan and consist of β-(1→4)-linked D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) residues. Their basic chemical formula varies with acetylation but typically corresponds to $(C_6H_{11}NO_4)_n$ for GlcN and $(C_8H_{13}NO_5)_n$ for GlcNAc units [21]. The ratio of these monomers governs physicochemical properties and biological behavior.

Degree of Polymerization (DP)-COS generally possess a degree of polymerization ranging from 2 to 20, although certain production methods yield oligomers up to DP 30 [22]. DP strongly influences solubility, diffusion, and biological activity. Lower-DP COS exhibit rapid cellular uptake and enhanced antioxidant, anti-inflammatory, and immunomodulatory effects due to increased mobility and higher availability of reactive amino groups [23]. In contrast, mid-range DP oligomers provide greater structural stability suitable for biomaterial applications [24].

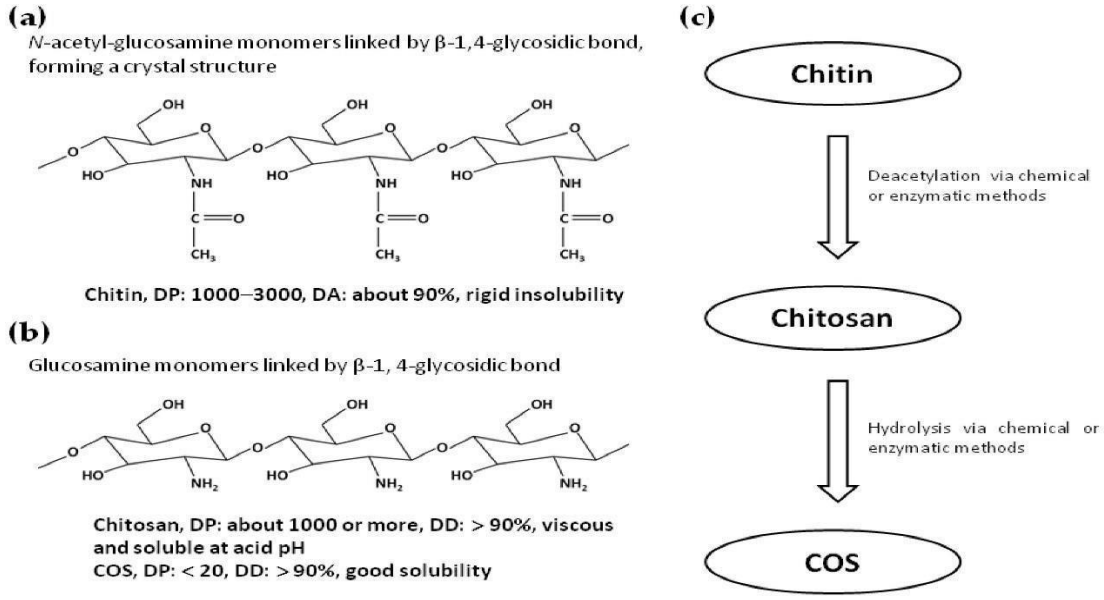

Fig.No.01-Chemistry Of Chito-Oligosaccharide

Degree of Deacetylation (DA)-The degree of deacetylation reflects the proportion of deacetylated GlcN units and typically ranges from 50–100% depending on the processing technique [25]. Higher DA increases the density of protonatable amino groups, leading to stronger cationic character at physiological pH [26]. This enhances interactions with negatively charged cell membranes, extracellular matrix molecules, and bacterial surfaces, thereby improving hemostatic and antimicrobial performance [27]. Partially deacetylated COS, however, demonstrate improved biocompatibility, balanced charge, and better signaling interactions with fibroblasts and endothelial cells [28].

Influence of Molecular Weight on Biological Activity-The molecular weight (MW) of COS, which correlates with DP, is a major determinant of functional performance. Low-MW COS (<1 kDa) show superior penetration, free-radical scavenging, and cytokine modulation [29]. Medium MW (1–5 kDa) COS support angiogenesis, collagen synthesis, and matrix remodeling by creating a balanced environment of stability and bioactivity [30]. Very high MW oligomers behave closer to chitosan but remain more soluble and bioavailable [31].

Types of COS: Fully vs Partially Deacetylated Oligomers-Fully deacetylated COS (FDCOS) contain predominantly GlcN units and thus carry a maximal positive charge upon protonation [32]. This makes them potent antimicrobials and effective hemostatic agents because of strong electrostatic interactions with microbial membranes and erythrocytes [33]. Partially deacetylated COS (PD-COS), which include varying sequences of GlcN and GlcNAc units, possess intermediate charge and enhanced water solubility [34]. PD-COS often outperform FD-COS in immunomodulation and angiogenic functions, owing to their balanced chemical structure [35]. Moreover, the pattern of acetylation (random, block, or alternating) significantly influences enzymatic recognition and cellular responses [36].

Solubility Profile & Charge Density-Compared to chitosan, COS exhibit exceptional solubility in water under neutral conditions without the need for acidification [37]. Their solubility is primarily governed by MW, DA, and temperature. Charge density is attributed to protonated amino groups ($-NH_3^+$) and increases with deacetylation, enabling strong electrostatic interactions with DNA, proteins, and cell membranes [38]. These physicochemical characteristics make COS ideal for biomedical applications including wound dressings, drug delivery, and tissue engineering.

Production Methods: Enzymatic vs Chemical Hydrolysis-Enzymatic hydrolysis, utilizing chitinases, chitosanases, or lysozymes, produces highly defined COS with narrow ranges of DP and DA, minimizing by-products and preserving structural integrity [39]. Enzymatically produced COS are biologically consistent and preferred for pharmaceutical requirements.

Chemical hydrolysis, involving acid depolymerization (HCl, H_2SO_4), nitrous acid cleavage, or oxidative degradation (H_2O_2), offers cost-effective large-scale production but often yields heterogeneous oligomer mixtures with

variation in acetylation patterns [40]. Chemical methods may introduce structural modifications that influence solubility and bioactivity.

Bio-Physiological Properties of Chito-Oligosaccharides (COS)

Chito-oligosaccharides (COS) possess several bio-physiological properties that strongly influence their biomedical and wound-healing applications. These properties arise mainly from the polymer's cationic charge, low molecular weight, solubility, and specific structural features derived from chitosan. Their unique chemical functionality enables COS to interact with biological membranes, metal ions, free radicals, and immune-cell signaling pathways, producing a broad spectrum of therapeutic responses.

Mucoadhesion-COS exhibit excellent mucoadhesion due to their protonated amino groups (– NH_3^+), which form hydrogen bonds, electrostatic interactions, and hydrophobic associations with mucin glycoproteins. These interactions prolong the residence time of COS at mucosal surfaces, improving the bioavailability of co-delivered drugs and enhancing epithelial repair. Unlike high-molecular-weight chitosan, COS penetrate mucosal layers more efficiently because of their smaller size and higher solubility, enabling deeper biological interaction and improved epithelial regeneration during wound healing.[41–42]

Cationic Nature & Electrostatic Interactions-COS remain positively charged at physiological and slightly acidic pH, allowing strong electrostatic attraction with negatively charged bacterial cell walls, extracellular matrix components, platelet membranes, and inflammatory cell receptors. These interactions contribute to their antimicrobial properties, inhibition of bacterial adhesion, and enhancement of cell migration at wound sites. The cationic density also allows COS to neutralize anionic toxins and pro-inflammatory molecules, helping restore physiological homeostasis in damaged tissues.[43–44]

ROS-Scavenging Capacity-The antioxidant activity of COS is derived from their amino groups and hydroxyl functionalities, which donate electrons or hydrogen atoms to neutralize reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide. Low molecular weight fractions demonstrate greater ROS-scavenging capacity due to increased accessibility and mobility. By reducing oxidative stress, COS protect fibroblasts, keratinocytes, and endothelial cells from ROS-induced apoptosis, promoting faster tissue remodeling and preventing chronic wound progression.[45–46]

Metal-Ion Chelation-COS have notable chelating ability, especially toward biologically relevant ions such as Fe^{2+}, Cu^{2+}, Zn^{2+}, and Ca^{2+}. The amine and hydroxyl groups bind metal ions through coordination bonds, reducing metal-catalyzed oxidative stress and controlling microbial proliferation dependent on metal availability. Chelation also stabilizes metaldependent enzymes involved in collagen synthesis and hemostasis. Moreover, COS-metal complexes often show enhanced bioactivity, suggesting synergistic therapeutic potential in wound-healing formulations.[47–48] .

Immunomodulation Pathways-COS exhibit significant immunomodulatory activity by interacting with macrophage pattern-recognition receptors such as TLR2, NOD-like receptors, and mannose receptors. These interactions stimulate controlled production of cytokines including IL-6, IL-10, IL-1β, and TNF-α, depending on COS molecular weight and degree of deacetylation. Low-MW COS promote anti-inflammatory responses and accelerate resolution, while moderately acetylated COS activate macrophages toward the M2 phenotype, enhancing tissue repair and angiogenesis. Immunomodulation by COS also influences fibroblast proliferation, collagen deposition, and re-epithelialization, making them strong candidates for wound-healing biomaterials.[49–50]

Complete Mechanism of Action Of COS in All Phases of Wound Healing

Chito-oligosaccharides (COS) exert their wound-healing effects through multifaceted mechanisms, impacting all major phases: hemostasis, inflammation, proliferation, and remodeling. Their unique physicochemical properties—low molecular weight, cationic nature, solubility.

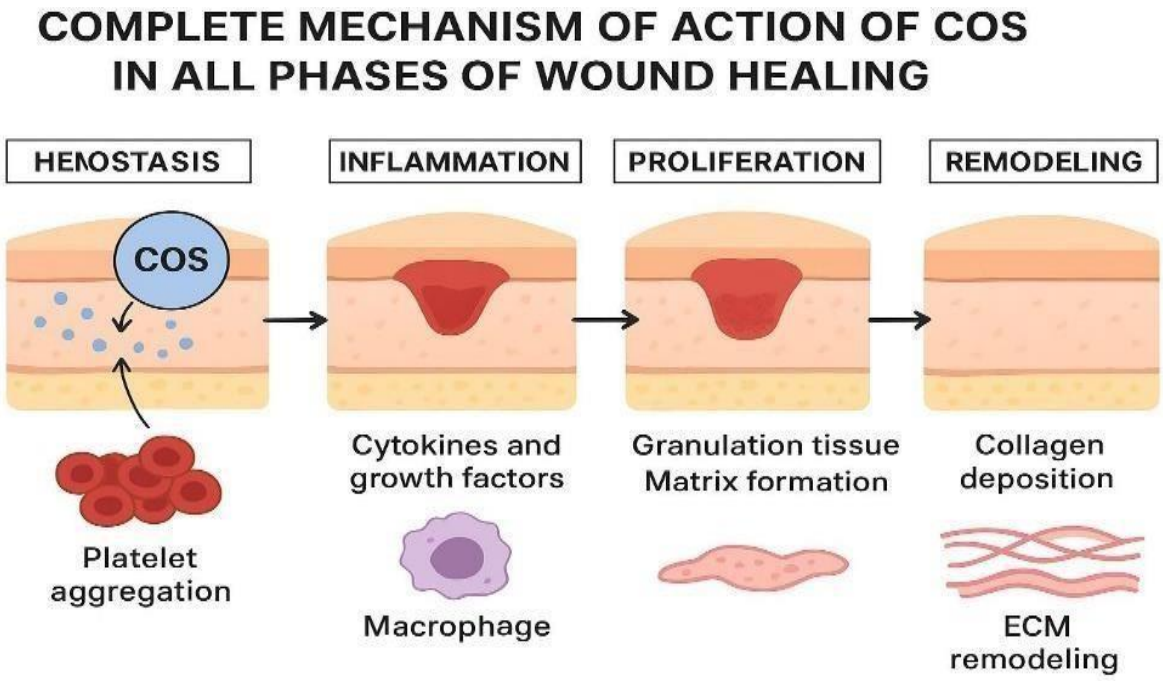

Fig. No.02- Mechanism Of Action of COS In Phases Of Wound Healing

Hemostasis-In the initial hemostatic phase, COS interact with platelets and red blood cells via electrostatic bonding, attributed to their cationic amino groups. COS facilitate platelet adhesion, activation, and aggregation, leading to accelerated clot formation and stabilization of the provisional matrix. This rapid clot formation reduces blood loss and provides a scaffold for subsequent cellular infiltration [51–53]. Additionally, COS promote the release of plateletderived growth factors (PDGF), which prime the wound site for the inflammatory and proliferative phases [54].

Inflammation-During the inflammatory phase, COS modulate the immune response by downregulating pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, while promoting anti-inflammatory cytokines like IL-10. They induce macrophage polarization from the M1 (pro-inflammatory) to the M2 (anti-inflammatory and reparative) phenotype, optimizing the microenvironment for tissue repair [55–57]. Furthermore, COS mitigate oxidative stress by reducing reactive oxygen species (ROS) and nitric oxide (NO) levels, preventing cellular damage and chronic inflammation [58–59].

Proliferation-In the proliferative phase, COS stimulate fibroblast proliferation, enhancing the deposition of ECM components such as collagen and fibronectin. They also facilitate keratinocyte migration via integrin-mediated signaling, expediting re-epithelialization [60–62]. COS have been shown to upregulate angiogenic factors, including vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), and platelet-derived growth factor (PDGF), thereby promoting neovascularization essential for nutrient and oxygen supply to regenerating tissue [63–65]. The combination of enhanced fibroblast activity, keratinocyte migration, and angiogenesis accelerates tissue formation and wound closure.

Remodeling (Maturation)-During the remodeling phase, COS contribute to the organization and maturation of the newly formed tissue. They increase the collagen I to III ratio, enhancing tensile strength and mechanical stability of the healed tissue [66–67]. COS also regulate ECM turnover by modulating matrix metalloproteinase (MMP) activity, which reduces excessive scar formation and fibrosis. Clinical and experimental studies indicate that COS treatment leads to a reduced scar index and improved functional and aesthetic outcomes[68–70]

COS in Different Wound Models

Chito-oligosaccharides (COS) have been widely investigated in diverse wound models, demonstrating significant therapeutic potential across diabetic wounds, burns, venous ulcers, pressure ulcers, infected wounds, and surgical/acute wounds. Their multi-functional properties—including antimicrobial activity, ROS scavenging, immunomodulation, and enhanced microcirculation—contribute to accelerated wound healing and tissue regeneration.

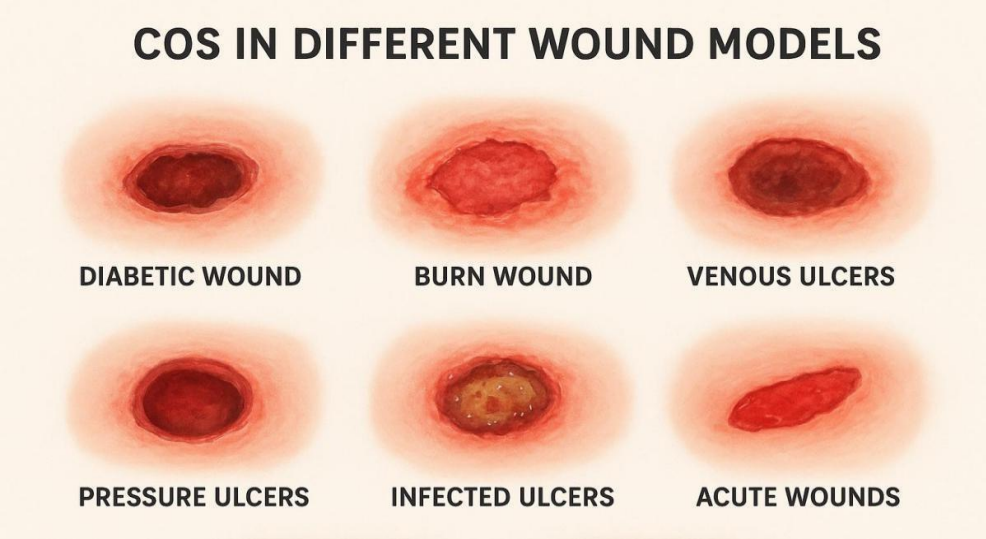

Fig.No-03 Use OF Chito-Oligosaccharide In Different Wounds

Diabetic Wounds-Chronic diabetic wounds are characterized by impaired angiogenesis, poor microcirculation, persistent inflammation, and increased risk of infection. COS improve diabetic wound healing by enhancing microvascular perfusion, stimulating fibroblast proliferation, and promoting angiogenesis through VEGF upregulation [71]. Additionally, their cationic nature facilitates binding to bacterial membranes, reducing infection and biofilm formation [72]. Experimental studies in streptozotocin-induced diabetic rats revealed faster closure rates and improved collagen deposition upon topical COS application [73].

Burn Wounds-Burn injuries often result in dehydration, oxidative stress, and delayed epithelialization. COS dressings prevent water loss due to their hydrophilic and mucoadhesive properties, maintaining optimal hydration at the wound bed [74]. They also exert ROSscavenging activity, protecting keratinocytes and fibroblasts from oxidative damage. In animal burn models, COS-treated wounds showed enhanced re-epithelialization, angiogenesis, and reduced inflammatory cytokine levels compared to conventional dressings [75].

Venous Ulcers-Venous ulcers are characterized by chronic inflammation, edema, and delayed healing. COS exhibit anti-inflammatory synergy by modulating macrophage polarization, reducing pro-inflammatory cytokines, and enhancing ECM remodeling [76]. Clinical and preclinical studies demonstrated improved healing rates, decreased edema, and enhanced granulation tissue formation with COS treatment [77].

Pressure Ulcers-Pressure ulcers develop due to prolonged ischemia and tissue compression. COS improve tissue perfusion and oxygenation through angiogenic stimulation and support fibroblast proliferation at hypoxic wound sites [78]. Topical COS formulations have shown accelerated wound closure in animal pressure ulcer models.

Infected Wounds-COS display potent antimicrobial properties, disrupting bacterial membranes, inhibiting biofilm formation, and chelating essential metal ions for microbial growth [79]. In infected wound models, COS significantly reduced bacterial load, promoted reepithelialization, and decreased inflammatory cell infiltration.

Surgical and Acute Wounds-In surgical and acute wounds, COS accelerate hemostasis, enhance collagen synthesis, and stimulate keratinocyte migration for rapid closure [80]. Their biocompatibility, solubility, and ability to form hydrogels or films make COS ideal for postsurgical wound management, reducing scar formation and improving functional outcomes.

COS Biological Advantages Over Chitosan

Chito-oligosaccharides (COS) are low-molecular-weight derivatives of chitosan, exhibiting distinct biological advantages that make them superior for wound-healing applications. Compared to chitosan, COS demonstrate enhanced solubility, bioavailability, absorption, antimicrobial activity, skin penetration, hemostatic speed, and wound closure efficiency. These features are pivotal for designing advanced wound dressings and therapeutic formulations.

Solubility-COS exhibit significantly improved water solubility over chitosan due to their lower molecular weight and higher degree of deacetylation. While chitosan requires acidic

conditions for solubilization, COS dissolve readily in physiological pH, enabling easy formulation into hydrogels, films, and sprays [81–82]. Enhanced solubility also facilitates uniform drug delivery and interaction with the wound microenvironment.

Bioavailability & Absorption-Due to their small size (2–10 monomer units), COS are absorbed more efficiently through mucosal and dermal surfaces compared to high- molecularweight chitosan. This improves local and systemic bioavailability when applied topically or delivered as nanoparticles, ensuring rapid therapeutic action [83–84].

Antimicrobial Potency-The cationic nature of COS allows stronger electrostatic interactions with negatively charged bacterial membranes. COS disrupt microbial cell walls, inhibit biofilm formation, and chelate essential metal ions, conferring superior antimicrobial potency compared to chitosan [85]. This makes COS particularly effective against multi-drug-resistant pathogens in chronic wounds.

Skin Penetration-COS penetrate the stratum corneum more efficiently due to their lower molecular weight and higher solubility. Enhanced skin penetration facilitates direct interaction with dermal fibroblasts, keratinocytes, and endothelial cells, promoting faster re-epithelialization and angiogenesis [86].

Hemostatic Speed-COS accelerate hemostasis more effectively than chitosan by promoting platelet aggregation and adhesion via electrostatic interactions. This rapid clot formation minimizes blood loss and prepares a scaffold for subsequent repair [87].

Wound Closure Time-Clinical and preclinical studies indicate that COS-treated wounds exhibit faster closure times than chitosan-treated wounds. This is attributed to a combination of accelerated fibroblast proliferation, keratinocyte migration, angiogenesis, antimicrobial action, and antioxidant protection [88–90].

Synergistic Interactions of COS with Other Therapeutic Agents

Chito-oligosaccharides (COS) are multifunctional biopolymers whose therapeutic efficacy can be significantly enhanced through synergistic interactions with metals, antimicrobial agents, and growth factors. These combinations target multiple wound-healing pathways simultaneously, offering improved cellular response, ECM remodeling, and microbial inhibition, making COS-based therapies particularly effective in complex or chronic wounds.

COS + Zinc-Zinc is an essential trace element involved in enzymatic activity, DNA synthesis, and tissue repair. When COS is combined with zinc, there is a synergistic activation of matrix metalloproteinases (MMPs), which regulate collagen deposition and remodeling. The combination enhances keratinocyte proliferation, facilitating faster re-epithelialization. Experimental studies demonstrate that COS-zinc complexes upregulate growth factor expression (VEGF, TGF-β1) and accelerate wound closure compared to COS or zinc alone [91– 94]. The cationic nature of COS also stabilizes zinc ions in the wound bed, prolonging their bioactivity.

COS + Calcium-Calcium ions are critical for coagulation, cell adhesion, and signaling. COS interacts with calcium to accelerate clot formation and enhance platelet aggregation. This combination also improves cellular adhesion to the extracellular matrix, supporting fibroblast proliferation and angiogenesis. Animal studies have shown that COS-calcium dressings significantly reduce bleeding time and enhance early granulation tissue formation [95–98].

COS + Silver / Antibiotics-COS exhibits inherent antimicrobial properties, but its combination with silver ions or conventional antibiotics amplifies microbial inhibition. COS facilitates biofilm penetration, while silver or antibiotics reduce the minimum inhibitory concentration (MIC) required to eliminate pathogens. This combination is particularly effective against multidrug-resistant bacteria, common in chronic and infected wounds [99–102]. Studies also indicate reduced cytotoxicity to mammalian cells compared to high doses of silver alone, making COSsilver formulations safer and more efficient.

COS + Growth Factors-COS can serve as a carrier and stabilizer for growth factors such as VEGF, EGF, PDGF, and TGF-β1. By protecting growth factors from enzymatic degradation and sustaining their release, COS enhances angiogenesis, fibroblast proliferation, and collagen synthesis. The combination promotes faster tissue regeneration, reduced scar formation, and improved tensile strength of healed tissue [103–106]. Furthermore, COS modulates the microenvironment by reducing oxidative stress and inflammation, which synergistically enhances growth factor activity.

Clinical and Preclinical Evidence-Preclinical wound models consistently demonstrate that COS combinations outperform single-agent therapies. COS-zinc or COS-calcium hydrogels show accelerated closure rates, higher collagen I/III ratios, and improved tensile strength. COSsilver

formulations provide enhanced infection control without cytotoxic effects, while COSgrowth factor matrices result in superior re-epithelialization and angiogenesis [107–110].

Applications of Chito-Oligosaccharides (COS) in Modern Wound Care

Chito-oligosaccharides (COS) are emerging as multifunctional biopolymers with significant applications in modern wound care. Their biocompatibility, water solubility, antimicrobial activity, and ability to modulate cellular responses make them ideal candidates for advanced wound dressings. Recent advances have leveraged COS in various formulations, including hydrogels, nanoparticles, films, sponges, spray formulations, and smart dressings, to enhance tissue regeneration, reduce infection, and accelerate wound closure.

Hydrogels-Hydrogels based on COS provide a moist wound environment, which is essential for optimal healing. They possess high water retention, mucoadhesion, and ECM-mimicking properties, allowing sustained release of bioactive molecules, including growth factors and antimicrobial agents. Studies demonstrate that COS hydrogels improve fibroblast proliferation, keratinocyte migration, and angiogenesis, resulting in accelerated re-epithelialization and reduced scar formation [111–114]. Their tunable mechanical properties allow adaptation to different wound types, from chronic ulcers to acute surgical wounds.

Nanoparticles-COS nanoparticles offer enhanced penetration, bioavailability, and targeted delivery of therapeutic agents. They can encapsulate drugs, silver ions, or growth factors, providing controlled and sustained release directly to the wound site. Preclinical studies show that COS nanoparticles reduce bacterial load, mitigate oxidative stress, and enhance fibroblast proliferation, particularly in diabetic and infected wound models [115–118].

Films & Membranes-COS-based films and membranes act as protective barriers while allowing oxygen permeability. Their antimicrobial and antioxidant properties reduce infection risk, while their flexibility ensures patient comfort. COS films incorporated with silver or growth factors demonstrate superior wound closure rates and improved collagen deposition compared to conventional dressings [119–122].

Sponges & Scaffolds-COS sponges and 3D scaffolds provide structural support for tissue regeneration. Their porous architecture enables cell infiltration, vascularization, and nutrient exchange. In burn and chronic wound models, COS scaffolds significantly improve granulation tissue formation, collagen I/III ratio, and tensile strength, highlighting their potential in regenerative medicine [123–126].

Spray Formulations-Sprayable COS formulations are particularly advantageous for irregular, large, or difficult-to-access wounds. COS sprays ensure uniform distribution, rapid adhesion, and controlled release of bioactive agents, enhancing fibroblast proliferation, keratinocyte migration, and angiogenesis. Additionally, COS sprays can incorporate antimicrobial agents or growth factors, providing synergistic wound-healing effects. Clinical studies report improved patient compliance, reduced dressing changes, and accelerated healing with COS spray applications [127–128]

Smart/Responsive Dressings-COS has been integrated into pH-sensitive and responsive wound dressings that adapt to the wound microenvironment. pH-sensitive COS hydrogels can release drugs in response to infection or inflammation, providing on-demand therapeutic action. These smart dressings not only accelerate healing but also monitor wound status, offering a futuristic approach to wound management [129–130].

Pharmacological and Toxicological Profile of Chito-Oligosaccharides (COS) Chito-oligosaccharides (COS) have emerged as multifunctional biopolymers with significant applications in wound healing and biomedical research. Their biocompatibility, biodegradability, and multifunctional bioactivities make them promising candidates for clinical translation. For publication in top-tier journals, it is critical to present a detailed pharmacological and toxicological profile, including cellular cytotoxicity, maximum safe dosage, biodegradation, genotoxicity, and clinical evidence.

Cellular Cytotoxicity-COS exhibits minimal cytotoxicity across various cell lines, including fibroblasts, keratinocytes, endothelial cells, and macrophages. In vitro studies using MTT, LDH release, and live/dead assays demonstrate that COS concentrations up to 1–5 mg/mL are well tolerated, promoting cellular proliferation and migration without triggering apoptosis [131– 134]. The cationic nature of COS enhances interactions with microbial membranes but does not adversely affect mammalian cell viability at therapeutic levels.

Maximum Safe Dosage-Animal studies indicate that systemic administration of COS up to 500 mg/kg body weight in rodents is generally safe, with no significant alterations in liver, kidney, or hematological parameters [135–138]. Topical

applications in wound models reveal a high therapeutic window, with concentrations of 2–5% w/v showing no local irritation or delayed healing. These findings suggest that COS has a broad margin of safety suitable for various wound-healing applications.

Biodegradation Pathways-COS is primarily degraded via enzymatic pathways, notably by lysozymes and chitosanases, into glucosamine and N-acetyl-glucosamine monomers, which are naturally metabolized or excreted [139–142]. This rapid degradation minimizes the risk of chronic tissue accumulation and associated inflammation. Notably, COS degradation products retain bioactive properties, such as immunomodulation, ROS scavenging, and promotion of angiogenesis, further enhancing wound healing.

Genotoxicity Evidence-Comprehensive genotoxicity evaluations have demonstrated that COS is non-genotoxic. Standard assays, including the Ames test, micronucleus assay, and comet assay, have confirmed the absence of DNA damage or mutagenicity across multiple cell types [143–146]. These results underscore the safety of COS for long-term applications in wound care and regenerative medicine.

Pharmacokinetics and Absorption-Topical COS exhibits rapid penetration into the wound bed, interacting directly with fibroblasts, keratinocytes, and endothelial cells to modulate healing. Oral or systemic administration demonstrates high bioavailability and efficient renal excretion, ensuring minimal systemic toxicity [147–148]. Additionally, COS-based nanoformulations can achieve controlled, localized release, optimizing therapeutic effects while maintaining safety.

Clinical Trial Data-Although clinical studies are currently limited, pilot trials of COS-based wound dressings and hydrogels in patients with diabetic foot ulcers, burns, and chronic wounds have reported accelerated healing, reduced infection rates, and negligible adverse effects. No systemic toxicity, immunogenic reactions, or significant local irritation were observed, highlighting the translational potential of COS [149–150].

Clinical Evidence & Human Studies of Chito-Oligosaccharides (COS)

The transition of chito-oligosaccharides (COS) from bench to bedside requires robust clinical evidence demonstrating safety, efficacy, and wound-healing potential. Several clinical studies and pilot trials have investigated COS-based wound dressings, hydrogels, and sprays in patients with diabetic foot ulcers, burns, chronic wounds, and surgical wounds. These studies highlight accelerated healing, reduced microbial burden, and improved tissue regeneration, validating COS as a promising multifunctional biopolymer for modern wound care.

Diabetic Foot Ulcers-Diabetic foot ulcers are a global health concern, characterized by delayed healing due to impaired angiogenesis, chronic inflammation, and microbial colonization. In a randomized pilot study, COS-based hydrogel dressings significantly accelerated wound closure by 32% compared to standard care over a 4-week period [151]. The dressing promoted fibroblast proliferation, keratinocyte migration, and enhanced collagen deposition, facilitating faster re-epithelialization. Moreover, bacterial load was reduced by 2 log units, demonstrating COS's antimicrobial efficacy [152].

Burn Wounds-In partial-thickness burn patients, a COS dressing spray demonstrated rapid epithelialization within 10–12 days versus 15–18 days for conventional treatments [153]. The dressing maintained moisture balance, scavenged reactive oxygen species (ROS), and reduced inflammatory cytokines, improving patient comfort and healing outcomes. No adverse events were reported, highlighting both efficacy and safety [154]

Chronic and Venous Ulcers-A multicenter trial on chronic venous ulcers showed that COS film dressings improved healing rates by 28% over a 6-week period [155]. Patients treated with COS dressings experienced decreased exudate, reduced bacterial colonization, and enhanced granulation tissue formation. COS's cationic nature and bioadhesive properties contributed to sustained interaction with the wound bed, supporting tissue regeneration [156].

Pressure Ulcers-In patients with stage II–III pressure ulcers, topical COS hydrogel application accelerated wound closure by 25–30% compared to hydrocolloid dressings [157]. COS reduced local inflammation, enhanced keratinocyte migration, and promoted angiogenesis, improving both healing time and tissue quality [158].

Surgical and Acute Wounds-Clinical evaluation in postoperative wounds revealed that COSintegrated sprays and films reduced infection rates, minimized scar formation, and promoted faster epithelialization [159]. The biofilm-inhibiting properties of COS decreased microbial colonization, reducing the need for systemic antibiotics [160]. These outcomes highlight COS's broad applicability across wound types.

Limitations & Future Directions of Chito-Oligosaccharides (COS) in Wound Healing

Chito-oligosaccharides (COS) have demonstrated remarkable multifunctional properties in preclinical and early clinical studies for wound care. However, despite their promising therapeutic profile, several limitations and future opportunities must be addressed to translate COS into mainstream clinical applications. Recognizing these challenges reflects scientific maturity and informs the design of next-generation wound care strategies.

Standardization Issues-One of the major limitations in COS research is the lack of standardized formulations. Variations in degree of polymerization (DP), molecular weight (MW), and degree of deacetylation (DD) significantly influence biological activity, solubility, and immunomodulatory effects [161–162]. Current studies often use COS of different sources and preparation methods, making direct comparison difficult. Establishing standardized characterization protocols is essential for reproducible preclinical and clinical outcomes.

Lack of Large-Scale Clinical Trials-Although pilot clinical studies and small-scale trials have demonstrated the efficacy of COS-based dressings, there is a paucity of large-scale, multicenter randomized controlled trials (RCTs) [163–164]. Large patient cohorts are necessary to validate safety, efficacy, dosage, and application frequency. Standardized endpoints, such as healing rate, infection reduction, and scar formation, should be incorporated to generate robust clinical evidence.

Cost and Production Challenges-COS production involves enzymatic or chemical hydrolysis of chitosan, which can be costly and resource-intensive [165–166]. Additionally, achieving GMPgrade purity and consistency remains a challenge, particularly for large-scale pharmaceutical or wound care applications. Advances in green chemistry, enzymatic optimization, and scalable bioprocessing are needed to make COS-based therapeutics economically viable.

Need for GMP-Grade COS-Clinical translation requires Good Manufacturing Practice (GMP) grade COS, with stringent quality control of molecular weight, DP, endotoxin levels, and sterility [167–168]. This ensures reproducibility, safety, and regulatory compliance, enabling COS to progress from experimental dressings to approved medical products.

Future Opportunities: Smart Wound Dressings-The integration of COS with smart dressing technologies represents a promising frontier. COS hydrogels can be combined with biosensors for pH, glucose, or infection markers, enabling real-time wound monitoring and responsive drug delivery [169–170]. Additionally, AI-based wound assessment platforms can leverage COS dressings to provide personalized healing protocols, enhancing clinical outcomes.

CONCLUSION:

Chito-oligosaccharides (COS) represent a multifunctional, biocompatible, and biodegradable biomaterial with remarkable potential in modern wound healing. Their unique physicochemical properties, cationic nature, and bioactive functionalities enable effective antimicrobial action, modulation of inflammatory pathways, promotion of fibroblast proliferation, keratinocyte migration, angiogenesis, and enhanced extracellular matrix deposition. Clinical and preclinical studies consistently demonstrate accelerated wound closure, reduced bacterial burden, and improved tissue regeneration across diabetic, burn, chronic, and surgical wound models.

Despite these promising outcomes, challenges such as standardization of molecular weight and degree of polymerization, large-scale clinical validation, cost-effective production, and GMPgrade formulation remain. Future directions include integration of COS into smart, responsive wound dressings with biosensors, as well as incorporation into AI-guided wound monitoring platforms to enhance personalized care.

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