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Chitosan Oligosaccharides as a Nanomaterial Platform: Biological Properties and Applications in the Biomedical and Pharmaceutical Fields

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Abstract

Chitosan oligosaccharides (COS) have been introduced as marine-derived biomaterials with potential health benefits and good water solubility properties. This study presents an overview of the promising nanomaterial platform for biomedical and pharmaceutical applications of COS. The health benefits of COS, primarily their antioxidant and protective effects, anti-inflammatory activity, antidiabetic properties, and cholesterol-lowering effects are discussed. Furthermore, the promising recent articles on specific topics such as drug delivery systems and nanobiomaterials, are highlighted.

Keywords: antioxidants, biomaterials, chitosan oligosaccharides, drug delivery systems, nanoparticles

Introduction

Nanomaterials have emerged as a platform for improving the functionalities of various compounds in different fields. Applying nanomaterials has several advantages, such as enhancing activity and properties, easy modification, and targeted activity [1]. Nanomaterials have been synthesized from a wide range of materials, such as polysaccharides, proteins, inorganic materials, metallic compounds, and others. Among these, polysaccharides are one of the promising materials due to their proven safety, cost-effectiveness, as well as biological and therapeutic activities [2]. Chitin and chitosan, as well as their oligomers, are being considered for applications due to their bioactive properties, non-toxicity, biocompatibility, and biodegradability [3, 4]. These marine-derived amino polysaccharides provide important structural and functional properties for various food, pharmaceutical, and biomedical applications. However, the use of chitin and chitosan is limited due to their high viscosity and low solubility at neutral pH, [5]. Chitosan oligosaccharides (COS) are the hydrolyzed forms of CS that have been introduced as potential biomaterials because they exhibit a wide range of biological activities and may have health benefits [6]. We hypothesized that COS have better capability than CS in promoting health due to their high solubility and remarkable biological activities. Several studies have shown that COS have anti-allergy, anti-arthritis, immuno-activating, neuroprotective, and other potential health effects, such as antioxidant, anticholesterol, antidiabetic, and antiinflammatory effects [7]. Therefore, COS have emerged as a biomaterial platform to synthesize nanomaterials.

Several review articles have focused on COS-based biomaterials or their specific applications [3, 8, 9]. Park and Kim [8] reviewed the biological activities of chitin and COS and their applications as drug delivery systems (DDS). Satitsri and Muanprasat [9] summarized the potential of COS as biomaterials applicable for treating a variety of diseases. Kumar and Kumar [3] studied the biomedical applications of COS, including drug and gene delivery and tissue engineering. The limitations of these studies include the absence of COS-based nanomaterial and the COS applications in the biomedical and pharmaceutical fields are limited to drug delivery and tissue engineering. A review focusing on COS-based nanomaterials is highly needed but is still lacking. Thus, in this study, recent articles on COS-based nanomaterials, biological properties, and their applications in biomedicine and pharmaceutics are highlighted.

Structure and Chemistry of COS

COS are a degraded form of CS, an amino polysaccharide that is commonly extracted from crustacean shells. COS consist of N-acetyl-D-glucosamine (GlcNAc) and glucosamine (Glc) units linked with β-1, 4 glycosidic bonds (Figure 1). Although their chemical structure is similar to CS, they have a lower molecular weight (MW) and degree of polymerization (DP) than CS, making them soluble in water and at neutral pH. Generally, CS with an MW and DP below 10 kDa and 20, respectively is considered as COS [6, 7]. However, those with an MW of 24 kDa and DP of 62 are still considered COS due to their water solubility [10]. Lower DP (DP of 2–12) COS are of great interest because of their potential health

benefits [11–13]. COS are positively charged from the free amino groups in the Glc units, which allows them to bind with negatively charged molecules and promote biological applications [14]. Moreover, COS have been produced by hydrolyzing CS using physical (microwave and ultrasonic treatments), biological (chitosanases and celluloses), and chemical (acid hydrolysis and oxidative degradation) methods [15] (Figure 2). COS with a defined MW, DP, degree of acetylation (DA), and acetylation pattern (AP), are desired because of their biological and physiological activities [16].

Biological Properties of COS

In this section, this study briefly highlights some of the important biological properties of COS that draw the attention of experts in the synthesis of nanomaterials. Refs [4, 7, 14, 18] are recommended for a more comprehensive review of the biological properties of COS. Unfortunately, the validation methods for the biological assays (antioxidant, antiinflamatory activity as well as antidiabetic, anticholesterol, and antibacterial effects) and authentication of the microorganisms used were not reported in all of the publications cited below. Thus, the reliability of the reported data may not be confirmed.

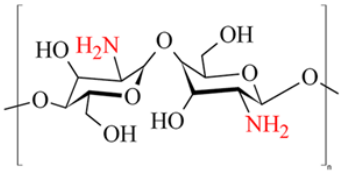

Figure 1. Structure of COS. Reproduced from [17] Under the Terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/).

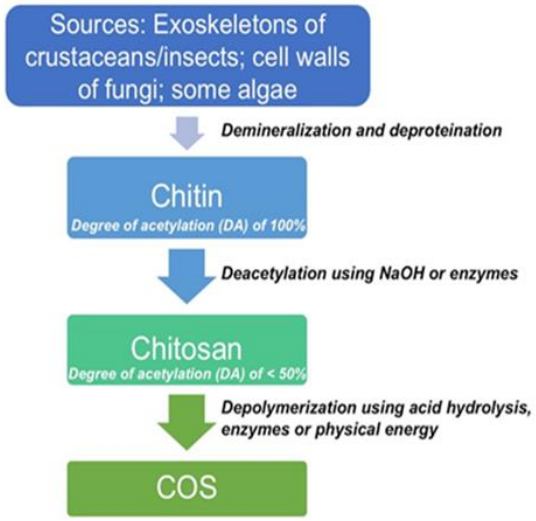

Figure 2. COS Production Method

Antioxidant and protective effects. Several *in vitro* studies have shown that COS possess strong antioxidant properties. Their free radical scavenging activity has been studied using the electron spin resonance method, where COS with an MW of 1–10 kDa show prominent scavenging activity on hydroxyl radicals, superoxide radicals, alkyl radicals, and 2,2-diphenyl-1-picrylhydrazyl radicals [19]. These are common free radicals with stronger scavenging activity on hydroxyl and superoxide radicals. The antioxidant activity of COS is associated with DP and depends on MW, DA, and the free radicals tested. In general, the higher DA of COS, the higher the antioxidant activity of COS, while medium MW COS (1–5 kDa) exhibits the highest antioxidant activity [11, 19].

The first study on the antioxidant and protective effects of COS in the cellular oxidizing system was reported by Kim *et al*. [20]. They discovered that low molecular COS (<1 kDa) suppress radicals and protects deoxyribonucleic acid (DNA) against oxidative damage in the B16F1 murine melanoma cell line. A correlation was detected between the presence of COS and the induction of the naturally occurring intracellular antioxidant glutathione (GSH). Moreover, several studies have been carried out using biological systems. These included a test on two biological oxidizable substrates, such as erythrocytes and bacteriophages, where two COS mixtures (<3 and <5 kDa), and low MW COS (<1 kDa) protected DNA against hemolytic damage [21]. It has also been reported that COS can protect Chang liver cells from oxidative damage induced by tert-butylhydroperoxide by inhibiting reactive oxidative species (ROS) production and lipid peroxidation [22].

A previous study reported that COS are potential neuroprotective agents for treating neurodegenerative diseases because of their ability to prevent glucose deprivation-induced cell apoptosis [23]. Similarly, COS are a potential biomaterial in the treatment of cardiovascular disease [24, 25]. COS protect human umbilical vein endothelial cells (HUVECs), which are commonly used to study the pathogenic mechanisms of cardiovascular diseases [24]. COS increase viability, and proliferative activity, and recover nuclear chromatin damage in HUVECs. COS (100–200 µg/ml) protected endothelial cells from oxidative damage similar to vitamin C (250 µg/mL) [24].

Several attempts have been made to improve the cellular antioxidant activity of COS by combining COS with certain antioxidants, such as phenolic compounds [26]. Covalently linked COS and gallic acid improve intracellular ROS scavenging activity and oxidative inhibition of DNA, proteins, and lipids in mouse macrophages (RAW 264.7 cells) and human chondrosarcoma (SW 1353 cells).

In animal studies, COS protect pancreatic islet cells from streptozotocin (STZ)-induced diabetes in rats. Moreover, COS ranging from 250 to 1,500 mg/kg body weight (b.w) in a dose-dependent manner enhanced the total antioxidant capacity, and superoxide dismutase activity, and significantly decreased serum malondialdehyde (MDA) levels. Based on morphological observations, COS protected the pancreas against STZ, where the greatest protective effect occurred with the medium dosage (500 mg/kg b.w of COS) [27]. Another study examined the protective effect of COS against sepsis, a systemic inflammatory response induced by infection. As a result, COS (100 mg/kg b.w) decreased MDA levels in the liver, lungs, and kidneys of mice, and increased GSH, and catalase activities. Furthermore, COS treatment improved the survival rate of mice after injecting a proinflammatory agent [28], and 500 mg/kg b.w COS restored serological parameters, including blood urea nitrogen, creatinine, and histology in rats after a paraquat injection [29]. COS have protective effects on intestinal integrity and are a promising novel anti-aging compound in the mouse model [30–32].

Despite the encouraging results of *in vitro* and animal studies, the antioxidant activities of COS have rarely been reported in clinical trials. One study reported the antioxidant activity of COS with an MW of 20 kDa and a degree of deacetylation (DD) of 95% [33]. In that study, 10 healthy subjects aged 23–35 years were given a single daily dose of 540 mg COS. After 4 weeks of treatment, human serum albumin (HSA) and total plasma antioxidant capacity were measured in a blood sample. HSA was used as the marker of oxidative stress in systemic circulation. The study showed that COS significantly lowered the oxidized albumin ratio by 18.6% ($p < 0.05$) and increased total antioxidant capacity by 44.2% ($p < 0.05$) after 4 weeks of treatment. They also examined the antioxidant properties of high molecular weight chitosan (HMWC) with an MW of 100 kDa [34] using a similar method. The results showed that HMWC supplementation significantly lower the oxidized albumin ratio by 12.5% ($p < 0.05$) and 11.8% ($p < 0.05$) after 4 and 8 weeks of treatment, respectively. Similarly, HMWC increased total plasma antioxidant capacity by 32.6% ($p < 0.05$) and 24.8% ($p < 0.05$) after 4 and 8 weeks of treatment, respectively. A clinical trial discovered that COS have stronger antioxidant activity than CS possibly due to the weak intra and inter-molecular hydrogen bonds.

Anti-inflammatory activity. Pro-inflammatory cytokines, such as interleukin (IL)-1 and tumor necrosis factor-alpha (TNF-α), cause inflammation. Therefore, several studies have focused on decreasing or blocking the release of pro-inflammatory cytokines using COS to reduce their biological activities [35] and exert antiinflammatory properties.

Studies on the antiinflammatory effects of COS have been carried out using various methods, and have been primarily based on inhibiting TNF-α, IL-1, and IL-6. In an *in vitro* study, 5 mg/mL COS with MW of 1–3 kDa significantly decreased the expression of inducible nitric oxide synthase (iNOS), which was associated with the inflammatory response [36]. It was reported that COS (0.05%–0.1%) recovered the levels of IL-6, and TNF-α proinflammatory cytokines in RAW 264.7 macrophage cells after injection of lipopolysaccharides (LPS), which increased the levels of TNF-α and IL-6. They also suggested that the antiinflammatory activity of COS was associated with the ability to affect TNF-α secretion [37].

An in-depth study on the molecular mechanisms of the anti-inflammatory activity of COS on LPS-induced RAW 264.7 macrophages was reported [38]. The inhibitory effects of a CS oligomer mixture (DD > 95%) on the overexpression of IL-6 and TNF-α were observed at the transcriptional and translational levels. COS (50–200 µg/mL) significantly suppressed the overexpression of IL-6 and TNF-α according to a reverse transcription-polymerase chain reaction analysis used to monitor mRNA transcription. A similar result was observed at the translational level, in which the production of IL-6 and TNF-α decreased significantly. COS have also shown suppressive effects on the phosphorylation of p38 mitogen-activated protein kinases (MAPKs), extracellular regulated kinases (ERK) 1/2, and c-Jun N-terminal kinases. These are the corresponding protein kinases for inflammatory signaling, inflammation-activating enzyme phosphatidylinositol 3-kinase, and protein kinase B (Akt), respectively. Furthermore, COS also inhibit the activation of nuclear factor-kB (NF-kB) and activator protein-1, which play an important role in inflammation [38].

Other studies have been carried out using HUVECs as a common tool to investigate vascular inflammation. The antiinflammatory activity of COS on LPS-induced HUVECs was slightly similar to LPS-induced RAW 264.7 cells. A COS (200 µg/ml) treatment suppressed transcription and translation of IL-6 by 35.5% and 60.9%, respectively compared with the LPS treatment. Furthermore, COS (200 µg/mL) inhibited the NF-kB protein by blocking NF-kB translocation from the cytoplasm to the nucleus and recovered the IkBα protein, which is an NF-kB inhibitory protein. The inhibitory effects of COS on p38 MAPK and ERK1/2 expression have been observed in LPS-induced HUVECs [39]. Moreover, COS inhibit the production and expression of adhesion molecules, such as vascular cell adhesion molecule 1 (VCAM-1), which promote vascular inflammation [40]. The antiinflammatory activity of COS on human THP-1 monocytes was also observed by reducing proinflammatory cytokines in a dose-dependent manner [41].

The first *in vivo* study of the anti-inflammatory activity of COS was reported by Fernandes *et al*. using a carrageenan-induced paw edema model, with two kinds of COS (1.2 kDa and 5.3 kDa) and DDs of 80%–85% [42]. They compared the activity with that of indomethacin (INN) as a positive control. Both INN and COS were injected into Balb/c mice 1 hour before the carrageenan injection, and the levels of paw edema were measured after 3 and 6 hours. Both COS at all concentrations (10, 30, 100, and 500 mg/kg b.w) decreased paw edema compared with the negative control, and the best activity was obtained at a dose of 500 mg/kg b.w [42]. The antiinflammatory activity of COS was compared with that of INN for up to 24 hours, however, the activity decreased for the next 24 hours. They concluded that the effects of COS depended on the MW, in which lower MW COS had better activity than higher MW COS. The proposed COS antiinflammatory mechanism occurs by inhibiting cyclooxygenase (COX) products, which are mediators of inflammation, by downregulating proinflammatory cytokines, such as TNF-α, IL-6, iNOS, and COX-2. The COS used in that study were considered to be safe as they did not damage the internal organs of the mice after treatment [42]. More recent *in vivo* studies have shown that COS are a potential therapeutic biomaterial to treat inflammation [30, 43–45].

Antidiabetic effect. Damage to the pancreatic β-cells has been reported to cause diabetic complications. Pancreatic β-cells must be protected because of their susceptibility to oxidative damage due to the low expression of antioxidant enzymes. An *in vitro* study showed that COS protected pancreatic β-cells from oxidative damage [27, 46] by preventing apoptosis in pancreatic islet cells (NIT-1). It was discovered that COS significantly improved the capability of antioxidant enzymes and increased the protective effect on pancreatic islet cells from oxidative damage [27]. COS can also amplify the INS-1 cells, increase insulin secretion by promoting glucose transporter (GLUT2) gene expression, and protect pancreatic β-cells from apoptosis induced by STZ [47].

Animal and cell studies have been carried out on the antidiabetic effect of COS. The first *in vivo* study on the antidiabetic effect of COS was conducted using STZ-induced diabetic rats [48]. The results showed that COS, with an MW of <1.5 kDa, successfully increased glucose tolerance and insulin secretion after 4 weeks of treatment. Furthermore, the COS lowered the glucose levels of diabetic rats and significantly decreased fed-triglyceride (TG) levels by 49%, indicating the ability to lower TG in diabetic rats. However, no significant change in glucose tolerance was observed with water containing 0.1% ascorbic acid [48].

Similar results were obtained in more recent studies by several authors [27, 47, 49–51]. COS with an average MW of 1.2 kDa were administered to STZ-induced diabetic rats at different concentrations of 250, 500, and 1,500 mg/kg b.w per day. After 60 days of treatment, 2-hour plasma, urine, and oral glucose tolerance tests were conducted. The best result was shown in the 500 mg/kg b.w concentration group, which decreased urine and plasma glucose levels to 16.14 mmol/L ($p < 0.01$) and significantly improved glucose tolerance with a blood glucose area under the curve of 68.7% [27]. Another study reported that COS improve oral glucose tolerance and decreases fasting blood glucose and insulin. In this study, a significant increase in liver glycogen and glucokinase was observed in the rats after the COS treatment. COS improved the pancreas-to-body weight ratio and minimized damage to the pancreas of diabetic rats [47]. A study on the antidiabetic effect of COS using lower dosages (5 mg and 10 mg/kg b.w per day) in diabetes alloxan-induced mice revealed promising antidiabetic activities at lower concentrations [51].

The first human clinical trials on the antidiabetic effect of COS were reported by Kim *et al*. [52]. As a result, Koreans with normal blood glucose levels and supplemented with 500 mg of COS had significantly lower postprandial blood glucose levels. This occurred 30 min after the meal by stimulating the action of insulin and inhibiting carbohydrate-hydrolyzing enzymes that decrease the release of glucose from the meal [52, 53]. COS supplementation also significantly decreases serum glucose levels after 12 weeks of supplementation [54]. COS effectively lowered the postprandial blood glucose levels of subjects with impaired glucose tolerance, fasting, and healthy glucose status [55].

Although the COS antidiabetic mechanisms have not been well-established, several mechanisms have been suggested. It has been proposed that COS protect pancreatic β-cells from oxidative damage due to their antioxidant activity. It has been suggested that pancreatic β-cells are strongly correlated with the incidence of diabetes [46]. One report showed that COS enhance the performance of pancreatic β-cells by promoting proliferation and the recovery of damage [27]. COS also maintain glucose homeostasis by lowering excess plasma glucose under diabetic conditions. This is due to their endocrine property of regulating the secretion of insulin and promoting the normal metabolism of plasma glucose. In addition, COS stimulate glucose to produce liver glycogen [47]. COS enhance insulin sensitivity by reducing the levels of tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6), and increasing adiponectin concurrently [50]. A recent study showed that COS inhibit digestion, transport, and uptake of glucose by inhibiting intestinal α-glucosidase and the glucose transporters, sodium-dependent glucose transporter 1 (SGLT1) and GLUT2, and enhances adipocyte differentiation as well as peroxisome proliferator-activated receptor γ (PPARγ) expression [49].

Anticholesterol effect. High blood cholesterol is a major risk factor for cardiovascular disease [56]. Therefore, it is important to maintain blood cholesterol at a normal level. One way to restore the blood cholesterol level is to use a cholesterol-lowering drug; however, adverse effects can make these drugs unfavorable [57]. CS has been promoted as a natural safe material to lower blood cholesterol [58]. COS have been increasingly studied due to their better solubility and biocompatibility compared with CS. COS have shown blood cholesterol-lowering activity in an animal model and a clinical trial.

A study using hyperlipidemic rats revealed that the supplementation with COS (250 and 750 mg/kg b.w) for 3 weeks significantly decreases rat plasma triglyceride (TG) and increases plasma high-density lipoprotein (HDL)-cholesterol by 29%–31% and 8%–11%, respectively. Furthermore, the lipid fractions were affected by COS. It was discovered that COS decrease plasma very low-density lipoprotein and low-density lipoprotein (LDL) fractions and increases plasma HDL fractions using protein liquid chromatography. COS also lower the portion of the main apolipoprotein in LDL, apoB48, and B100. One experiment showed that COS do not affect plasma apoA-I, which is a major HDL apolipoprotein, indicating a major role in stimulating maturation or interfering with HDL catabolism. More importantly, COS significantly enhance the activity of lecithin cholesterol acyl transferase, an enzyme responsible for regulating the HDL cholesterol level. It has been suggested that COS affect lipid regulation, lipoprotein profiles, and regulatory enzymes. However, COS do not significantly reduce LDL-cholesterol levels [57].

In contrast, one study reported that supplementing mice with COS for 4 weeks significantly decreased total and LDL-cholesterol levels but did not affect the HDL cholesterol level [59]. They also investigated the effect of COS on reverse cholesterol transport (RCT), which promotes the secretion of cholesterol into bile acids, leading to increased cholesterol excretion through the feces. An isotope-labeled cholesterol-tracing assay was used to evaluate RCT in mice, and several genes and proteins associated with cholesterol transport such as scavenging receptor class type I (SR-BI), cholesterol 7α-hydroxylase (CYP7A1), ATP-binding cassette transporters (ABCA1/ABCG1), and LDL-receptor (LDL-R) were observed by western blot. This treatment increased the expression of SR-BI, which regulates the transport of cholesterol from the plasma to the liver without affecting the expression of ABCA1/ABCG1. These are the main cholesterol transporters, indicating that COS are partially related to enhanced hepatic SR-BI expression. Moreover, the accumulation of ^{3}H-cholesterol in the bile and feces of mice indicates that COS improve the expression of CYP7A1, which is an important gene in bile acid synthesis. The LDL-cholesterol-lowering effect of COS is due to their ability to increase the expression of the LDL-receptor. COS are a safe lipid-regulating treatment because they do not affect the metabolism of other lipids or morphology in mice [59]. In more recent studies, COS lowered cholesterol levels by enhancing the expression of hepatic LDL-R and SR-BI, CYP7A1, liver X receptor alpha, and PPAR-α and facilitated the conversion of cholesterol to bile acids [60,61]. However, the cholesterol-lowering effect of COS is slightly lower than that of CS [62]. In addition, COS have been proposed to be nutraceuticals for treating obesity due to their ability to control weight gain, improve dyslipidemia, and inhibit hepatic lipid accumulation [63–65].

Only one human study has reported on the anticholesterol activity of COS. Nineteen healthy men (11 smokers and 8 nonsmokers) received 500 mg COS of MW < 1 kDa and a DD of 90% orally twice daily. It was concluded that 1 g per day of COS for 6 weeks significantly lowered the total cholesterol and LDL-cholesterol levels of the participants compared with those at baseline. This finding shows that COS play an important role in changing the cholesterol metabolic pathway due to their ability to bind lipids and bile acids [66, 67].

Antibacterial activity. The antibacterial activity of COS has been reported by several studies, in which the growth of gram-positive bacteria, such as *Staphylococcus aureus*, with a low minimum inhibitory concentration of 1.13 µg/mL, was inhibited [68]. COS with an MW < 50 kDa, DD of 88.76%, and a concentration of 0.25% effectively inhibited the growth of *E. coli*, which is a gram-negative bacterium. The inhibitory effect of COS against *E. coli* increases with decreasing MW and vice versa [69]. In a recent study, the antibacterial activity of COS was tested against four gram-positive bacteria, such as *S. aureus* ATCC 43300 and 25923, *Bacillus subtilis*, *B. cereus,* and eight gram-negative bacteria, including *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Vibrio cholerae*, *Shigella disenteriae*, *Enterobacter agglomerans*, *Prevotella melaninogenica*, and *Bacteroides fragilis.* The results showed that COS were effective in inhibiting the growth of all of the bacteria tested. Additionally, the antibacterial activity of COS was better than that of chitin and CS. COS penetrated the bacteria cell wall, whereas chitin and CS only flocculated the bacteria. This observation shows that COS can act as a bactericidal agent, while chitin and CS merely act as bacteriostatic agents [70]. However, a different result has been reported, in which CS had better antimicrobial activity than COS [71]. Moreover, the possible mechanisms for the antibacterial activity of COS include flocculation of bacteria, preventing nutrients from entering the cell by forming a polymer membrane, electrostatic interaction with the components of the cell surface of the bacteria, and penetration into the cell wall of the bacteria [69, 70, 72].

Safety of COS

Human lymphocytes are an appropriate model for evaluating the biocompatibility and safety of a compound, as they play an important role in the immune system. Thus, the toxicity of COS was evaluated on human lymphocytes [73]. It was concluded that a concentration > 0.07 mg/ml was cytotoxic in a dose-dependent manner, but no genotoxic activity was observed. Another study demonstrated that no subacute toxicity was observed in rats given 2,000 mg/kg b.w./day, showing that COS supplementation was safe [74]. Qin *et al*. (2006) indicated that short-term (30 days) supplementation of rats with COS is safe because no adverse effects were observed after a 3 g/kg b.w/day treatment [75]. Similarly, COS do not affect DNA, plasma membrane integrity, motility, and morphology of sperm cells, suggesting the absence of acute toxicity [76]. COS supplementation was safe in a clinical trial [54]. Moreover, COS-based nanoparticles (NPs)(500 μg/mL) have relatively low toxicity *in vitro* [77]. However, more studies are needed to ensure the safety of COS-based nanomaterials in clinical studies because nanosized materials can be toxic to the body.

Synthesis of COS-based Nanomaterials

COS conjugates and micelles. COS-based conjugates are formed by linking COS with another compound through physical, chemical, or enzymatic processes [78] to endow COS with intended characteristics and improve the biological activities of COS. COS have been conjugated with proteins, inorganic compounds, polymers, and small molecules [78–81] using chemical methods. An amine-reactive crosslinker is widely used because the conjugation reaction is straightforward and cost-effective. This includes N-hydroxysuccinimide esters (NHS) and carbodiimide (EDC) [82], which are used to directly conjugate carboxylates to primary amines. EDC is also used with NHS to improve the conjugation reaction and create a more stable intermediate [83]. In rare cases, COS-based conjugates can be formed by simple mixing with the intended compounds followed by drying [81]. COS-based micelles are among the most popular nanomaterials used for several applications, such as DDSs. Micelles are self-assembled nanomaterials formed from amphiphilic molecules. The size of the micelles is 5–100 nm [84]. Furthermore, COS-based micelles are formed by covalently joining fatty acids as hydrophobic compounds to hydrophilic COS [85, 86] using EDC and NHS as crosslinkers.

COS Nanoparticles. COS NPs are among the most popular nanomaterials and have been fabricated by several bottom-up methods, such as emulsion, desolvation, and ionic crosslinking/electrostatic interactions/polyelectrolyte complexation [1]. In the emulsion method, the W/O surfactant acts as a template to fabricate the COS NPs. In addition, crosslinking agents are usually used to strengthen the integrity of the NPs. However, the drawbacks of this method are the additional washing of the surfactant and the use of toxic crosslinkers. The most popular method for making COS NPs is ionic crosslinking, in which the COS and anionic small molecule crosslinkers, such as tripolyphosphates, are mixed in an aqueous solution [5]. The mixing allows the electrostatic interactions between them, and COS are organized into spherical NPs. This method is popular due to its mild and easy reactions and it is free of toxicity. COS NPs have also been fabricated by polyelectrolyte complexation [87] using small molecules, proteins, and anionic polysaccharides. More stable COS NPs with additional properties have been synthesized through hydrophobic and electrostatic interactions. However, the drawback of this method is the relatively large size of NPs (> 150 nm) and the yield significantly depends on the magnitude of the interactions between the mixed compounds [88]. The desolvation method is also used to make COS NPs. This method is a simple coacervation or phase separation, in which macromolecular aggregation is induced by solvated molecules [1]. Similar to the emulsion method, a surfactant is frequently used to stabilize the NPs suspension.

COS as supporting materials. COS are widely used as supporting material to fabricate nanomaterials due to their inherent biological properties. Fabrication of hydrophobic nanomaterials, such as carbon- and metallic-based nanomaterials, requires the use of a hydrophilic compound for solubilization during the preparation of nanomaterials [17, 89–91]. COS can support such nanomaterials based on the formation of NPs and improved bioactivity. The benefits of COS properties, such as high water-solubility, low viscosity, antimicrobial, and anticancer, have attracted their use as supporting nanomaterials in several studies [14]. In addition, they are cost effective, abundant, and biocompatible and can be used in the biomedical and pharmaceutical fields. The general synthetic method for COS as supporting nanomaterials is by physical mixing or blending with other components to form COS-based nanocomposites with unique properties. A COS solution and various components are uniformly blended, followed by the sol gel transition and formation of the nanocomposite [66].

Applications of COS-based Nanomaterials

Drug delivery systems. A DDS is an excellent tool for delivering bioactive compounds, such as drugs, to the desired target. A DDS also aids in the long-term stability of the bioactive compound, which has added to popularity during the last two decades. COS-based nanomaterials have been used as delivery systems for a wide variety of compounds, such as small molecule drugs, proteins,

nucleic acids, and antioxidants [92–95]. Moreover, COS-based micelles have been designed as carriers of methotrexate (MTX), a drug used to treat inflammation and cancer [96]. In this system, MTX was covalently conjugated with COS to form micelles with particle sizes of 135–237 nm. In that study, indomethacin-conjugated COS NPs (IDM-COS NPs) were synthesized for tumor-targeted drug delivery [83] using doxorubicin, the anticancer drug as a cargo. Imaging and *in vivo* studies revealed that IDM-COS NPs have great potential to prolong the circulation time of doxorubicin, enhance anticancer activity, and improve tumor-targeting ability. Another COS-based nanomaterial was synthesized to overcome multidrug resistance in breast cancer cells. Nanohybrids consisting of silica NPs and copolymers of COS, polyethyleneimine, and folic acid have been designed to deliver paclitaxel and short hairpin RNA [97].

COS-based nanomaterials have great potential as protein delivery vehicles. A previous study reported using COS-heparin NPs (COS-hep NPs) to deliver cytokines, which are small proteins responsible for cell interactions [98]. They used stromal cell-derived factor-1α and vascular endothelial growth factor as cytokine models and discovered that COS-hep NPs were significantly stable under physiological conditions and effectively protected and delivered cytokines for tissue regeneration. Another COS-based nanosystem was developed to deliver small interfering RNA (siRNA) and Vanin-1, which is a pantethine that catalyzes the hydrolysis of pantetheine into cysteamine and vitamin B5 [99]. The nanosystem consists of COS lactate, a P3-peptide, and polyethylene glycol, and was designed for adipose-targeted delivery. The results showed that the nanosystem was effective in attenuating fat mass loss (lipolysis).

COS-based nanomaterials have been developed to deliver nucleic acids, such as DNA and siRNA, due to their cationic nature. Redox-responsive COS-octadecylamine NPs have been developed to deliver DNA specifically used to treat hepatitis B diseases [100]. The NPs were about 250 nm and effectively released DNA into the cytoplasm (Figure 3). Other studies have shown that these nanosystems are efficient in delivering siRNA for treating cancer cells and protecting siRNA from biodegradation [101].

Recently, COS nanomaterials have been proposed for novel ocular delivery systems [102] because COS as a bulk material prevent oxidative stress and inflammation of the retina [103]. Nanohybrid systems consisting of nanostructured lipid carriers (NLC) and COS have been designed to deliver small molecule drugs to the eye [104]. These nanosystems were significantly effective in prolonging precorneal retention and enhancing ocular bioavailability, corneal permeability, and ocular mucoadhesive properties [104, 105]. Recent progress in the application of COS-based nanomaterials for DDS is summarized in Table 1.

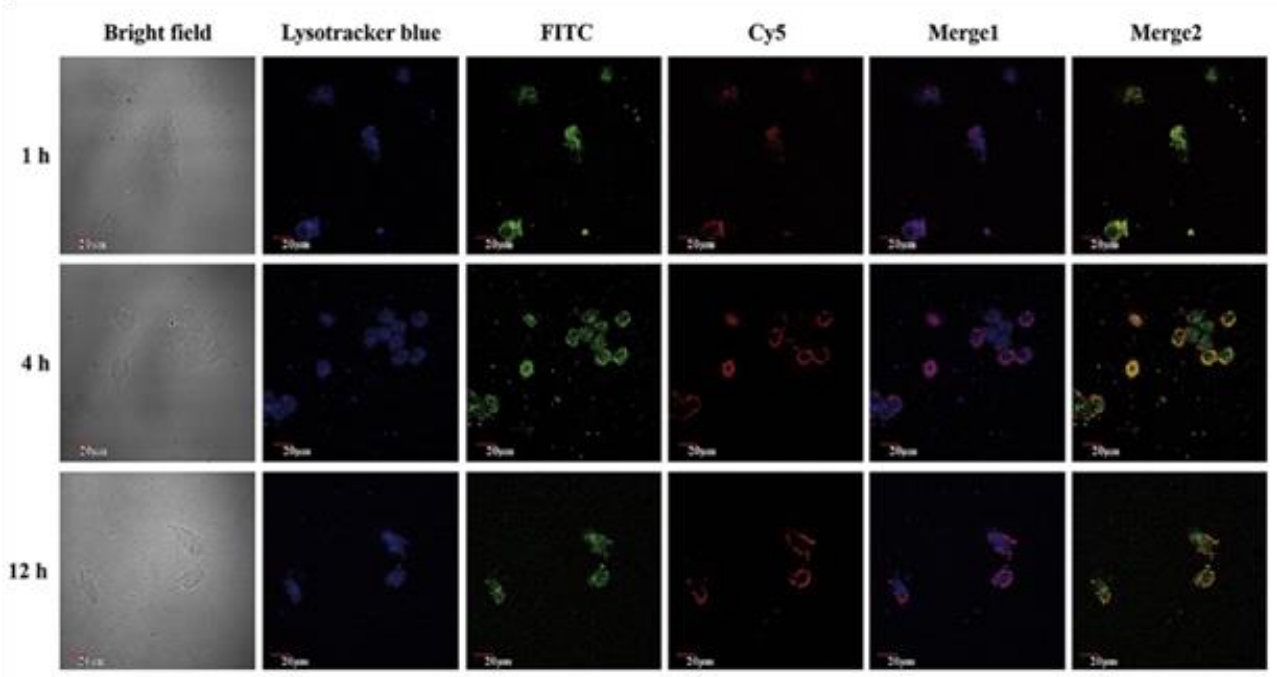

Figure 3. Intracellular Trafficking of COS-Octadecylamine NPs. DNA Escaped from the Lysosomes and was Released into the Cytoplasm, as Indicated in Merge 1 and 2. Lysotracker Blue, FITC, and Cy5 Label Show Lysosomes, COS-based NPs, and DNA, Respectively. Merge 1 Shows the Merged Images of Red and Blue Channels, while Merge 2 Shows the Merged Images of the Red and Green Channels. Reproduced from [100] under the Terms of the CC BY-NC-ND License (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Table 1. Recent Progress in the Application of COS-based Nanomaterials for DDS

Nanomaterials	Type of Nanomaterials	Cargo	Key Findings
COS-g-stearic acid	Micelles	Doxorubicin (DOX)	Redox-responsive release of DOX via breakable disulfide bond
COS-g-stearic acid	Micelles	DOX	Effective *in vitro* and *in vivo* inhibition effect on tumor and cancer stem cells
COS-indomethacin	Nanoparticles/ conjugates	DOX	Enhanced cytotoxicity of DOX Prolonged circulation in the blood of mice
Phenylboronic acid-Pluronic F127-COS	Micelles/ conjugates	DOX	Phenylboronic acid lower IC50 value on B16 cells Reduced DOX toxicity to the normal organs
Self-assembly of COS conjugates and mesoporous silica nanoparticles	Nanocomposites	DOX	A pH triggered charge-reversal nano system Synergistic effect in tumor inhibition and lower cardiotoxicity
Kartogenin conjugated COS and dicarboxylated pluronic F127	Nanospheres	Kartogenin and diclofenac	Thermo-responsive nanosystem with dual drug release for osteoarthritis treatment Effective in reducing inflammation and regenerate cartilage
Vitamin E-conjugated COS/Arginine-glycine-aspartic acid peptide (RGD)-conjugated D-alpha tocopheryl polyethylene glycol (PEG) 1000 succinate	Mixed micelles	Paclitaxel (PTX)	Sustained PTX release properties and high cytotoxicity Effective in killing U87MG tumor in mice
Folic acid-conjugated COS/magnetic halloysite nanotubes	Nanocomposites	Camptothecin	Enhanced cellular uptake due to folic acid and COS
COS-vanilin imine	Micelles	Genistein	Dual pH responsive nanomicelles via pH sensitive benzoic imine and COS protonation
Sialic acid-conjugated COS and biphasic calcium phosphate	Biomimetic nanoplatform	Methotrexate (MTX)	New nanoformulation-based strategy for rheumatoid arthritis
Podophyllotoxin conjugated COS-g-stearic acid	Micelles	Podophyllotoxin	Enhanced cellular uptake and cytotoxicity *in vitro* on glioma cells
Reducible hexadecanol COS micelles coated with hyaluronic acid and polyethylene glycol mixed with Fe_3O_4 nanoparticles	Multifunctional micelles	Gambogic acid	Enhanced cellular uptake and fast drug release Redox/pH dual responsive drug release
Covalent polyplex between cationic micelles of COS-conjugated polymer and negatively charged oligomeric hyaluronic acid	Multifunctional nanoparticles	Curcumin	Mitochondrial targeting and charge-reversal ability Effectively localized in mitochondria and effective tumor reduction in mice

Table 1. ***Continue***

Nanomaterials	Type of Nanomaterials	Cargo	Key Findings
Phycocyanin coated COS-dithiopropionic acid-curcumin	Multifunctional nanoparticles	Curcumin	Redox sensitive via disulfide bond between curcumin and COS Enhanced cellular uptake by active targeting via biotin receptor
Stearic acid-conjugated COS	Micelles	Emodin	Improved antitumor activity of emodin *in vivo*
COS-valylvaline-stearic acid	Micelles	Dexamethasone	Effective *in vitro* and *in vivo* ocular delivery Comparable to a positive control (Cequa, which is approved by FDA)
Pluronic F127 hydrogel and COS-hyaluronic acid nanoconjugates	Hydrogel/ Conjugates	Gallic acid	Better release profiles of gallic acid in neutral pH Low toxicity of the system on human cell keratinocytes for concentrations up to 20 μg/mL
Desalted duck egg white-COS conjugate	Conjugates	Calcium	Promoting calcium bioavailability and improving gut health *in vivo*
Oleic acid-conjugated COS-zinc and PLA-PEG-PLA copolymer micelles	Nanocomposites	Insulin	Slow controlled release of insulin Preserving and stabilizing insulin *in vivo*
P3-peptide COS lactate and PEG	Nanoparticles	Vanin-1	Vanin-1, a pantetheinase, regulate lipolysis and adiposity
Spermine-modified COS-g-stearic acid	Micelles	DNA	Promoting the escape of DNA release from endolysosome Transfection ability of micelles is comparable with Lipofectamine 2000 and polyethyleneimine (PEI) 25K
COS-bovine serum albumin (BSA) conjugate	Nanocomplexes	DNA	High *in vitro* transfection ability and good cell viability
COS-ss-octadecylamine	Nanoparticles	DNA	Redox-responsive COS-based nanoparticles for anti-hepatitis B therapy, with effective transfection ability and lower cytotoxicity
Galactosylated COS-octadecylamine	Micelles	DNA	Effective *in vitro* and *in vivo* gene delivery for hepatitis B Glutathione responsive and hepatocyte-targeting with no noticeable hepatotoxicity or systemic toxicity
Folic acid-modified PEG-COS lactate	Nanoparticles	siRNA	High efficiency to PDAC (pancreatic ductal adenocarcinoma) cells No toxicity *in vitro* and *in vivo* was observed
COS	Nanocomplexes	siRNA	COS with high DP (more than 50) produce smaller particle sizes and more stable complex
Folic acid-COS and silica nanohybrids	Nanocomposites	PTX and short hairpin (shRNA)	Synergistic effect and effective to tackle multi drug resistance

*No study reported authentication of the cell lines used

Wound healing. Wound healing is a complex physiological process involving a series of cellular and molecular events [120]. The potential of COS in wound healing is due to their biological activities, such as antimicrobial, antiinflammatory, immune-enhancing, cell proliferation, and adhesion-promoting activities [121], as well as good biocompatibility and cost-effectiveness. Therefore, several studies have fabricated COS-based nanosystems to accelerate wound healing. One example is a nanofiber composite, consisting of silver NPs, COS, and poly (vinyl alcohol) [120]. This system effectively promoted wound healing by increasing hydroxyproline (hyp) and collagen contents and activating the TGFβ1/Smad signaling pathway (Figure 4). Another nanocomposite was fabricated from halloysite, a natural nanostructured clay mineral, and COS [122]. This nanocomposite had a good *in vitro* biocompatibility on normal human dermal fibroblast and enhanced *in vitro* fibroblast motility, cell proliferation, and migration. The wound healing ability of the nanocomposite was significantly better than that of halloysite or COS separately. A new nanocomposite consisting of mesoporous glass NPs and COS-carboxymethyl starch nanocomplexes was recently developed to control surgical hemorrhaging [123].

Sensors and imaging. The use of nanomaterials for sensor and imaging applications has gained interest in recent studies. One class of materials with the capability for the development of sensors is a water-soluble responsive polymer. Similarly, COS have attracted attention in the development of sensor and imaging tools because of their responsive, biodegradable, and biocompatible properties. Sensors based on metallic NPs, such as gold, silver, palladium, and COS, have been devised to accurately detect H_2S and iodide. Additionally, noninvasive photo imaging for cancer therapy has been developed [125–127], in which COS were used as a stabilizer for NPs. Furthermore, targeted cancer imaging and photothermal therapy have been performed using fluorescence molecule-conjugated COS [128], where the COS were conjugated with the ZW800-1 near-infrared fluorophore. This system is effective for targeted tumor imaging and phototermal treatments (Figure 5). Other approaches using carbon-based nanomaterials, such as graphene oxide and silica NPs, were designed to develop sensors for detecting cholesterol and heparin [91, 129]. A paper-based colorimetric sensor for detecting glucose and uric acid has also been developed using COS as the color-forming reagent [130]. The results showed that COS enhanced the stability of the colorimetric signals and improved the clarity of the signal at the detection spot.

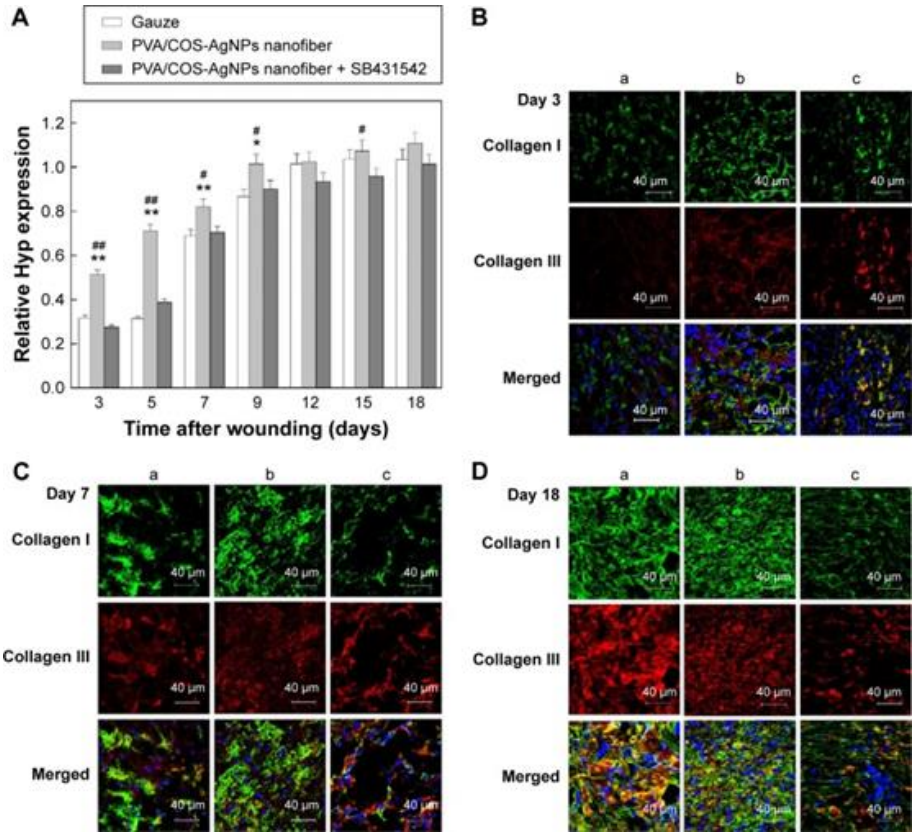

Figure 4. Hyp Content (A) and Immunofluorescent Staining of Collagen I and III (B–D) After Treatment with COS-based Nanofibers. Reproduced from [124] Under the Terms of the Creative Commons Attribution-Non Commercial License (http://creativecommons.org/licenses/by-nc/3.0/).

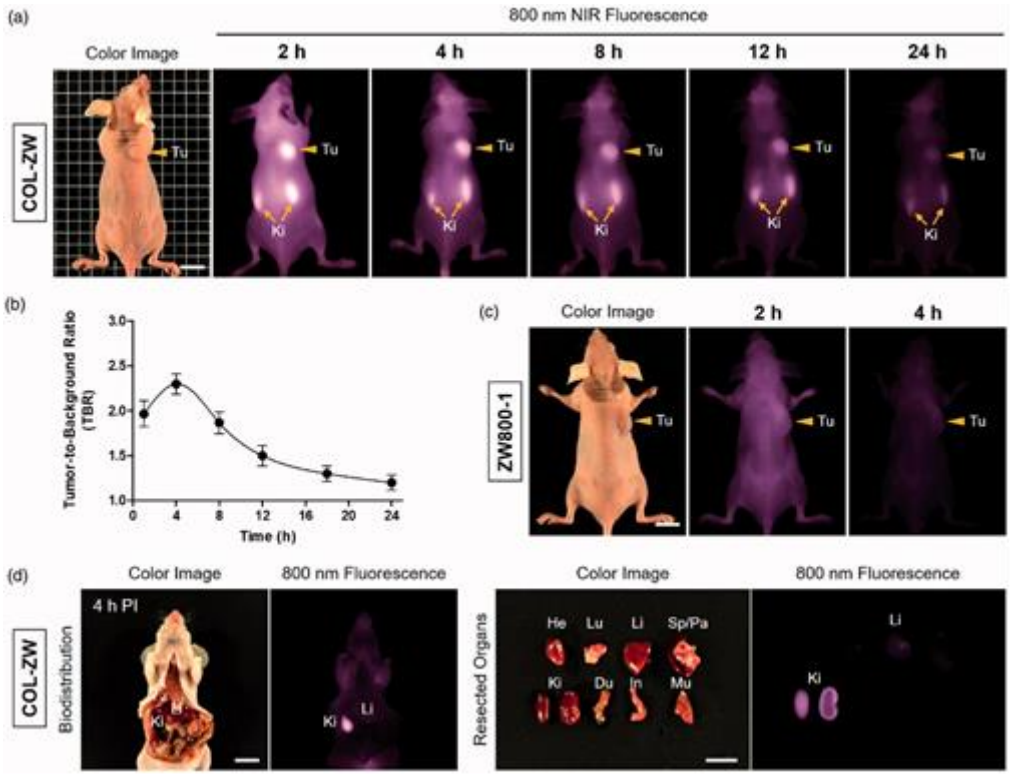

Figure 5. ***In vivo*** **Tumor-targeting Ability of COS-composite NPs, as Shown in (a). COS-composite NPs Specifically Target the Tumor, as Indicated by the Fluorescence Signals (White). Tumor is Indicated by the Tu Arrow. The Intensity of the Fluorescence Signals Decreased Over Time (b). Control is Shown in (c). Biodistribution of COS-composite NPs is Presented in (d). Reproduced from [128] Under the Terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/).**

Conclusions and Perspectives

COS are a promising material to synthesize polysaccharide-based nanomaterials as they are cost-effective and exhibit a wide range of biological activities. Furthermore, COS have beneficial health effects on the human body, such as a cholesterol-lowering effect, antidiabetic, antiinflammatory, and antioxidant activities. The biological activities of COS depend on the MW and DD. COS with a lower MW tend to have better biological activities. Some studies have shown that DP is significantly important in providing biological and therapeutic effects.

Although there has been great progress on COS-based nanomaterials for biomedical and pharmaceutical applications in the past decade, some concerns and obstacles need to be addressed. One concern is the production of COS as a biomaterial. COS have heterogeneity in molecular structure as a biopolymer, i.e., there are differences in DP, DD, MW, and the type of polymer chain structure, which affects the reproducibility and the quality of the synthesized nanomaterials. The varying molecular structures of COS also hinder the large-scale production and commercialization of the synthesized nanomaterials. Therefore, an advanced COS production method that minimizes the heterogeneity in the molecular structure is needed.

As most of the raw materials to produce COS are from crustaceans and seafood, their use could induce allergenicity and immunogenicity. This is another concern for the successful commercialization of COS-based nanomaterials for biomedical and pharmaceutical applications. One way to tackle this problem is by using noncrustacean-based raw materials, such as fungi. Thus, the use of fungal COS is encouraged in the future, although production costs are relatively higher.

In addition, few studies have been conducted on the safety of COS-based nanomaterials, and clinical trial research is rare. Thus, it is anticipated that more research in this area should be a priority in the future. Moreover, with the advancement of modification technology, COS derivatives-based nanomaterials could be seen as potential research topics in the future.

In summary, COS-based nanomaterials had excellent performance in *in vitro* and animal studies as a DDS, in wound healing, and as sensors. However, the effectiveness and the efficacy of COS-based nanomaterials for human use remain unknown due to

limited information from clinical trials. Also, for having reproducible results that can be replicated by other researchers, validation methods (chemical- and biological-assays) and the authentication of the cell lines and microorganisms should be reported in detail.

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