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Review Article

Chitosan Nanocomposites as Wound Healing Materials: Advances in Processing Techniques and Mechanical Properties

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ABSTRACT

This review discusses the increasing potential of chitosan nanocomposites as viable materials capable of targeting these debilitating factors. This review focuses on various techniques used to process chitosan nanocomposites and their mechanical properties. Chitosan nanocomposites are regarded as highly effective antimicrobials for the treatment of chronic wounds. Chitosan nanocomposites, such as chitosan/polyethylene and oxide/silica/ciprofloxacin, demonstrate efficient antibacterial activity and exhibit no cytotoxicity against Human Foreskin Fibroblast Cell Lines (HFF2). Other studies have also showcased the capacity of chitosan nanocomposites to accelerate and improve tissue regeneration through increment in the number of fibroblast cells and angiogenesis and reduction of the inflammation phase. The layer-by-layer technique has benefits, ensuring its suitability in preparing chitosan nanocomposites for drug delivery and wound dressing applications. While the co-precipitation route requires a cross-linker to achieve stability during processing, the solution-casting route can produce stable chitosan nanocomposites without a cross-

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linker. By using the solution casting method, fillers such as multi-walled carbon nanotubes (MWCNTs) and halloysite nanotubes (HTs) can be uniformly distributed in the chitosan, leading to improved mechanical properties. The antibacterial effects can be achieved with the introduction of AgNPs or ZnO. With the increasing understanding of the biological mechanisms that control these diseases, there is an influx in the introduction of novel materials into the mainstream wound care market.

Keywords: Chitosan nanocomposite, mechanical properties, processing techniques, tissue regeneration, wound healing

INTRODUCTION

Chitosan is a natural co-polymer of the deacetylated derivative of chitin units (β-(1→4)-linked D-glucosamine) as well as the non-deacetylated units (N-acetyl-D-glucosamine) (Islam et al., 2017; Liu et al., 2018). It is widely studied owing to its multifaceted applications and multitasking ability, besides its significant benefits, including biodegradability, biocompatibility, and non-toxicity (Singh et al., 2013; Islam et al., 2013). Chitin is the second most abundant biopolymer after cellulose, and its application in wound treatment is an incredible breakthrough, which is by virtue of its excellent attributes (Dai et al., 2011). To accelerate the healing process, the wound dressings, which have a crucial part in the wound healing niche, must possess certain important features, such as the ability to prevent bleeding and dehydration of the wound, ensure a suitable moist environment, protect the wound against external contamination, allow gas permeation and fluid exchanges, absorb exudates from the wound area, thermal isolation, biocompatibility, non-toxicity, and a non-allergenic profile (Zhong et al., 2010; Gu et al., 2009). Chitosan nanocomposites with various reinforcements, fabricated through simple and economically viable techniques, are one of the most used wounds dressing materials due to their enhanced and exceptional wound healing capability (Figure 1).

Based on these features, Lu et al. (2012) processed chitosan-polyvinyl alcohol (PVA)/graphene nanofibers using the electrospinning technique and investigated the prospects of these membranes in healing skin wounds in mice and rabbits. They discovered that the wound areas decreased significantly after five days, and a complete recovery was achieved at the end of 10 days. On the other hand, the wound areas were still evident when membranes without graphene were employed. Aguzzi et al. (2014) produced chitosan/montmorillonite nanocomposites loaded with silver sulfadiazine for a similar purpose. Through microstructural analysis, it was revealed that the silver sulfadiazine was successfully loaded into the nanocomposite structure. X-ray diffraction (XRD) analysis detected no free drug in the composite. It shows that intercalated nanocomposites can be produced with uniform drug and/or polymer molecule distribution.

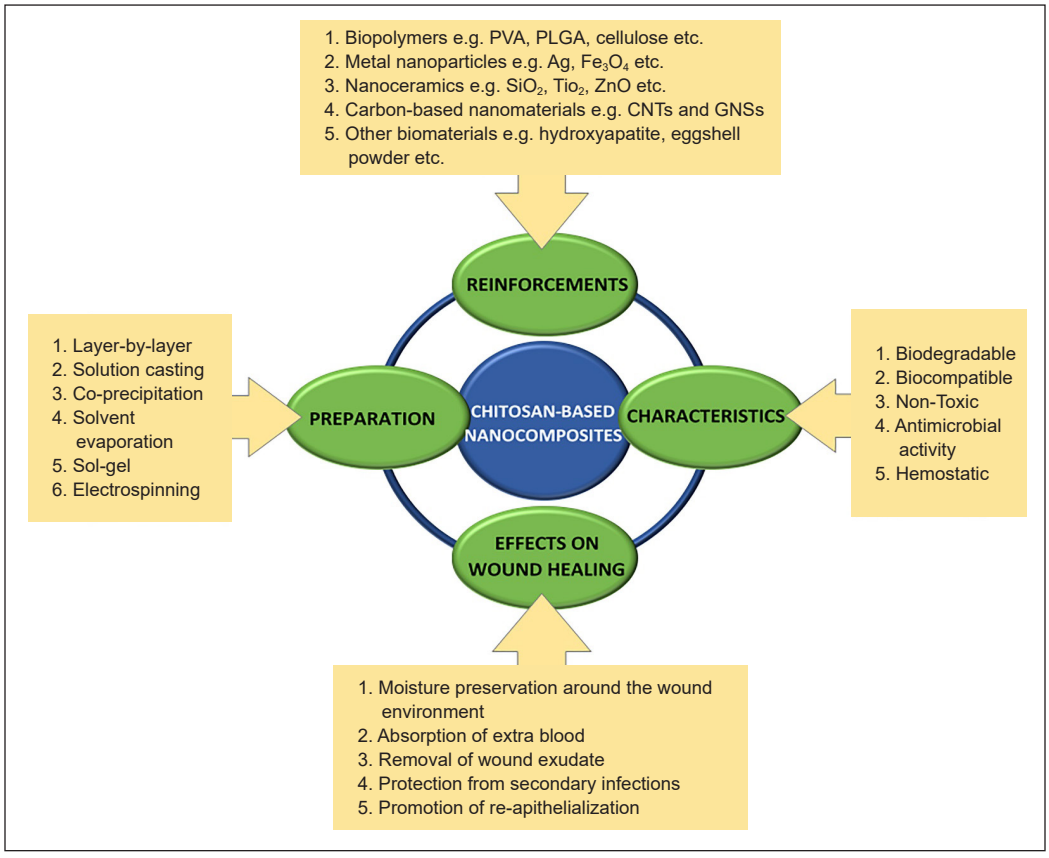


Figure 1. Overview of chitosan nanocomposite

In a similar study, Lu et al. (2017) produced a microporous chitosan-Ag/ZnO composite dressing that demonstrated enhanced antibacterial and wound healing activities. Both the surface and interior of the microporous chitosan-Ag/ZnO dressing possessed interconnected porous structures with a pore size in the range of 50–150 µm. After investigating for several days, the authors further observed that the in vivo wound healing ability of the CS-Ag/ZnO-0.5 composite surpassed those of ZnO ointment gauze, CS, and CS-ZnO-0.5 composites. Furthermore, compared to other types of dressing, the application of CS-Ag/ZnO-0.5 composite dressing resulted in smoother-grown skin with small scabs (Figure 2). In addition, an investigation of the antibacterial activity of the CS-Ag/ZnO-0.5 composite dressings on mice wounds was conducted, and only a small number of bacterial colonies were observed, which was owed to the outstanding antibacterial property of silver (Ag) and zinc oxide (ZnO).

Li et al. (2019) investigated collagen/chitosan gel composite enhanced with Oligoarginine (CPP: Cell-Penetrating Peptide). They discovered that the gel composite successfully suppressed *Staphylococcus aureus* growth, reflecting its excellent wound

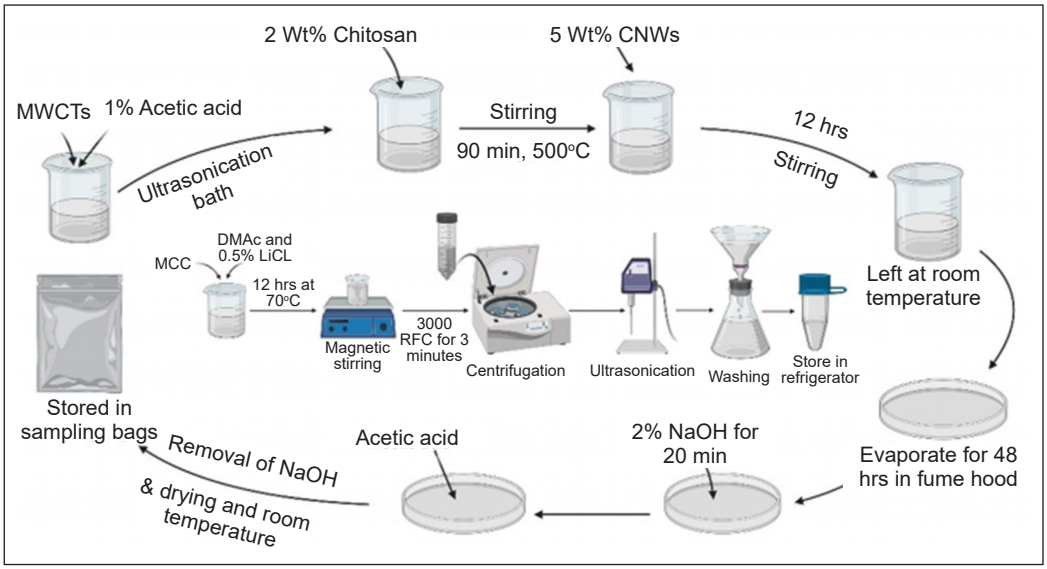


Figure 2. Schematic illustration of preparation of CS/CNWs/MWCNTs nanocomposites (Thou et al., 2021)

healing capability without substantial cytotoxicity. Furthermore, the results of the histopathological analysis revealed that the collagen/chitosan/CPP gel composites had great potential in promoting cutaneous wound healing by enhancing granulation tissue formation, increasing collagen deposition, and promoting angiogenesis in the wound tissue. In another study, chitosan and protein isolate composite hydrogels were developed for controlled carotenoid delivery and wound healing (Hamdi et al., 2020). The authors also developed models to investigate the vivo healing ability of the composite hydrogels in rats. It was observed that using the composite hydrogels loaded with carotenoids as topical patches accelerated the wound healing process and ultimately achieved full healing.

In another investigation, it was reported that biocomposite films containing chitosan, polyvinyl alcohol, polyvinyl pyrrolidone, and HMDACS (hexamethylene 1, 6-di(aminocarboxysulfonate)) cross-linker exhibited excellent antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, making the biocomposite films a promising wound dressing material (Rahmani et al., 2020). In a related study, a composite dressing composed of collagen, chitosan, and alginate (CCA) exhibited no substantial cytotoxicity and remarkably promoted hemocompatibility (Xie et al., 2018). The authors concluded that the CCA composite dressing could prevent seawater immersion and enhance wound healing, in addition to having reliable biosecurity.

Selecting the best route to develop chitosan nanocomposites is crucial to ensure their structural reliability, performance integrity, and resultant properties. Due to their solubility in acidic media, several techniques have been proposed to process stand-alone and reinforced chitosan nanomaterials and enhance their inherent properties. Furthermore,

these materials should have excellent mechanical properties to ensure their sustainability and ability to perform under various working conditions. This review highlights the research progress related to the processing techniques of chitosan nanocomposites and their mechanical properties.

PROCESSING ROUTES FOR DEVELOPING CHITOSAN NANOCOMPOSITES

As the demand for chitosan nanocomposites in various applications continues to increase, researchers have proposed various processing techniques to meet these demands. The selection and suitability of these fabrication techniques depend on the industrial requirement, cost-effectiveness, simplicity, and reproducibility. The commonly used techniques for processing chitosan nanocomposites are comprehensively discussed in the following sub-sections.

Layer-By-Layer Method

The layer-by-layer (LBL) is a simple, cost-effective, and multipurpose technique. In this technique, substrates combine and modify properties of various materials to achieve specific unique functions (Gulaczyk et al., 2003). The materials are deposited layer by layer using substrates that have strong interactions with them, which makes the technique attractive for processing nanocomposites (Podsiadlo et al., 2007). The layers of deposited materials interact via electrostatic interactions, covalent bonding, and bio-specific interactions. Furthermore, using the LBL technique ensures the efficient distribution of particles and interaction between components while reinforcing the chitosan nanocomposite matrix with other materials to achieve the desired structure and function.

Siqueira et al. (2006) assembled metallophthalocyanines with chitosan (Ch) in nanostructured films through an electrostatic LBL route. The procedure comprised the preparation of chitosan and metallophthalocyanine solutions at room temperature and the fabrication of the films. The silicon and Au-coated glass substrates were dipped alternately in the chitosan and metallophthalocyanine solutions and subsequently rinsed and dried under nitrogen (N2) gas. The fabricated films possessed high stability with 0.8 and 0.75 reversible redox peaks and could detect low dopamine concentrations. The redox peaks reflected the high anodic and cathodic currents in the bilayer. It was concluded that this technique enables the incorporation of various nanoparticles and metallophthalocyanine molecules into LBL films and the modification of the films. This technique may find applicability in bioanalytical and other fields.

In a similar study by de Mesquita et al. (2010), cellulose nanowhiskers (CNW) and chitosan (CS) composites were assembled on a glass or quartz substrate via the LBL route. It was concluded that the processing route could be extended and used to develop

various biopolymers, which can fabricate novel biobased nanocomposites applicable in biomedical fields such as drug delivery. This conclusion was drawn from observing the homogenous distribution and strong interaction between the cellulose whiskers and chitosan nanocomposites. In addition, the LBL technique ensured that the hydrogen bond and electrostatic interaction between CS and CNW were matched for proper adherence, which is one of the important features of a wound dressing material.

Li et al. (2013) developed chitosan (CS) and cellulose in nanocrystal form (CNs) on an amorphous poly (ethylene terephthalate) substrate (glass slides and silicon wafers) via the LBL technique and analyzed their oxygen-barrier properties. The CS/CNs nanocomposite was revealed to have a low oxygen permeability coefficient and a less superior oxygen barrier property compared to the composites with the LBL and inorganic coatings. Another significant advantage of the LBL technique is the prospect of designing/producing high-performance materials with high precision. It also allows the analysis of the interface between components to understand better the resultant properties of the multilayered thin films (Pinto et al., 2017). For example, the analysis of the morphology and thickness of the interface between fabricated carbon dots and chitosan biopolymer suggested that both parameters can be tailored and reproduced by controlling the biopolymer solution and the number of dips while employing the LBL technique.

Huang et al. (2012c) investigated the effect of the number of deposited bilayers and the composition of coating bilayers on their morphology and antibacterial activities. The bilayers were produced via the LBL technique, in which the negatively charged electrospun cellulose acetate (CA) fibrous mats were combined with positively charged lysozyme (LY)–chitosan–organic rectorite (OREC). It was reported that the various deposition conditions significantly influenced the morphology of the LBL film-coated fibrous mats, especially when the bilayers were doubled to increase deposition thickness. It created big junctions, and the addition of OREC significantly impeded the growth of *Escherichia coli* and *Staphylococcus aureus*. OREC is known to possess high absorption ability. Therefore it can easily absorb onto surfaces and suppress the proliferation of bacteria. The authors concluded that the scope of the LBL approach could be expanded and exploited by developing and analyzing the structure and surface properties of 3D LBL materials, which could be suitable for various applications such as antimicrobial wound dressing and tissue engineering.

In a recent study, Huang et al. (2019) deposited chitosan (CS) and tannic acid (TA) composite on non-fibrous mats to study its antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The antibacterial activity of the LBL cellulose-CS/TA mats was observed to increase significantly with the increasing number of coatings. Furthermore, the antibacterial activity against *S. aureus* was reported to be higher due to the higher sensitivity of the bacteria as compared to that of *E. coli*. Conclusively, the LBL technique

was suggested as a prominent and reliable technique to fabricate films for drug delivery application and as wound dressing materials as it does not alter the substrates' size and shape. Furthermore, the LBL route ensures efficient contact and interaction between bacteria and LBL film coating. Apart from the non-flexibility of chitosan polymer, which was regarded as a drawback in the processing of LBL films with clay nanosheets (Liu et al., 2009), the LBL technique can be considered an appropriate method for preparing chitosan nano-multilayer films as it eliminates the disadvantages that include low stability and poor absorption.

Solution Casting Method

Solution casting is a popular technique due to its simplicity and versatility. It has been extensively employed to produce antibacterial chitosan films for biomedical applications. Tripathi et al. (2011) stated that this technique is superior because the film can be formed without a cross-linker. In preparing chitosan-silver oxide nanocomposite film via solution casting, silver-oxide nanoparticles were prepared by adding trisodium citrate into silver nitrate ($AgNO_3$) to obtain black precipitates before filtering and rinsing. The prepared silver-oxide solution was added to the chitosan solution (1% w/v in 1% acetic acid) and stirred for two hours. The mixed solution was later cast onto glass plates and dried for 48 hours to obtain the desired composite films. The analysis results suggested that the silver oxide nanoparticles in the chitosan matrix improved the antimicrobial activity; hence, it was deemed suitable for wound healing.

In a much more recent and related study that aimed to explain and comprehensively describe the versatility of the solution casting technique, Thou et al. (2021) investigated the influence of functionalized multi-walled carbon nanotubes (f-MWCNTs) and untreated multi-walled carbon nanotubes (Un-MWCNTs) on the structure and properties of chitosan matrix mixed with cellulose nanowhiskers (CS/CNWs) produced via solution casting. The step-by-step processing of CS/CNWs/MWCNTs composite was carried out by adding 0.5% f-MWCNTs and Un-MWCNTs to the acetic acid solution. First, the mixture underwent dispersion treatment for one hour, and then 2 wt.% chitosan powder was added. Next, the mixture was stirred before 5 wt.% CNWs were added, after which a homogenous mixture was formed through continuous stirring. Subsequently, the homogenous mixture was cast on a petri dish and left to dry until a nanocomposite film was formed. The schematic description of the process is presented in Figure 2. The results revealed that f-MWCNTs and un-MWCNTs were adequately dispersed in the CS/CNWs aqueous solution. Nevertheless, the f-MWCNTs-reinforced composite possessed better mechanical properties, which was in line with the findings by Celebi and Kurt (2015).

Darder et al. (2006) stated that one of the important factors to be considered in evaluating the properties of nanocomposites is the dispersion of nanofillers, and a suitable

solution casting technique can result in a good dispersion in the chitosan matrix. To further support this statement, Huang et al. (2012a) introduced sepiolite into chitosan poly (vinyl alcohol) (PVA) nanocomposite via solution casting. The reinforcement with sepiolite was observed to have significantly enhanced the mechanical properties of the nanocomposite due to the intermolecular hydrogen bonds between the sepiolite, PVA, and chitosan. Similarly, Huang et al. (2012b) prepared nanocomposite films by reinforcing chitosan (CS) and poly (vinyl alcohol) (PVA) with halloysite nanotubes (HTs) via solution casting. With the aid of a field emission scanning electron microscope (FESEM) to analyze the morphology of the samples, it was revealed that the processing technique could guarantee better miscibility, which in turn enhances the mechanical properties and water resistance of the nanocomposite film. Another notable benefit of the solution casting technique is that it can produce circular and translucent membranes containing chitosan, as reported by De Silva et al. (2013).

The excellent water vapor and oxygen permeability of biocompatible chitosan and poly (vinyl pyrrolidone)/Nanocellulose (CPN) composite were discovered by Poonguzhali et al. (2017). The composite could maintain a moist environment over the wound bed, which was also expected to prevent further contaminations. Patel et al. (2018) prepared Lupeol-entrapped chitosan-gelatin hydrogel (LCGH) films via solution casting. After the equilibrium water content (EWC) and water vapor transmission rate (WVTR) were determined, the results revealed that the prepared LCGH films were ideal for wound healing with an EWC of 85.40% and WVTR of 2228 ± 31.8. In addition, the antibacterial activity of the Lupeol was maintained, as assessed via the disc diffusion method. It shows that this processing route has flexibility, and the LCGH film can be a suitable delivery system for the sustained release of Lupeol for enhanced wound healing.

The interaction between the positively charged chitosan molecules and negatively charged microbial cell membranes can disturb the metabolism of bacteria strains. At the same time, the large surface area of the nanocellulose facilitates the adsorption of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In a more recent study, Rahmani et al. (2020) prepared chitosan (CS), polyvinyl alcohol (PVA), and polyvinyl pyrrolidone (PVP) composite via solution casting. The electrostatic forces between CS and charged microbial cell membrane inhibited the activity of *Escherichia coli* and *Staphylococcus aureus*. The flexibility of the solution casting technique plays an important role in the modification of chitosan nanocomposites in the way that the thickness of the composite films can be controlled by customizing the amount of solution used during processing (Fan et al., 2012). While solution casting is a popular technique to prepare materials suitable for wound healing and food packaging applications, its major shortcoming is the possibility of retaining solvents during processing. Therefore, it is mostly not recommended for tissue engineering applications due to the possibility of toxicity of the produced composite (Boccaccini & Ma, 2014).

Co-Precipitation Method

Co-precipitation is another method that has been utilized by researchers, with significant achievements in producing reinforced chitosan nanocomposites with desired functionality. Unlike the solution casting technique, the co-precipitation method utilizes a cross-linker to achieve stability during processing. The technique has been recognized as an accomplished and economical means to mass-produce nanomaterials (Chen et al., 2008; Raoufi, 2013). In a study by Yamaguchi et al. (2001), the co-precipitation method was used to prepare chitosan/hydroxyapatite (CS/HAp) composites. In the initial preparation stage, 3 wt.% chitosan aqueous solution was prepared by dissolving chitosan powder in 1 wt % acetic acid and adding 8.5 wt % H_3PO_4 solution. Subsequently, the chitosan/H_3PO_4 solution was mixed with $Ca(OH)_2$ suspension to obtain a slurry, which was then aged for 24 hours. The procured precipitate was filtered, washed, and compressed into a cylindrical form under a pressure of 20 MPa. Varma et al. (1999) explored several processing routes to develop CS/Hap composite. They stated that the co-precipitation technique's drawbacks included the powder mixtures' dissatisfactory homogeneity and the noticeable inflammation of the samples. Nonetheless, this technique enabled the production of samples with mechanical flexibility and significant homogeneity.

The route was further used by Sreedhar et al. (2007) to develop HAp/Carboxymethyl chitosan (CMCh) nanocomposites to explain the excellent thermal stability of the nanocomposite, which resulted from the strong interaction that existed within the prepared samples. Agglomerate-free Fe_3O_4-gold-chitosan nanoparticles with improved morphology were highlighted in the study by Salehizadeh et al. (2012). The excellent morphology was achieved using formaldehyde as a cross-linker to ensure stability and improve gelation properties in the nanocomposites. Zhang et al. (2012) produced chitosan-coated magnetite (Fe_3O_4/CS) nanocomposites, where the co-precipitation reaction was facilitated by applying a magnetic field. Analysis of the magnetite crystallinity revealed that the magnetic field improved the morphology of the synthesized Fe_3O_4/CS nanocomposites. The study by Li et al. (2016) further emphasized the benefits of cross-linker in the co-precipitation technique. The results proved the improvement in the properties and conductivity of methanol fuels by adding glutaraldehyde cross-linker into chitosan, reinforced with quaternized poly (vinyl alcohol). The cross-linker significantly reduced the particle size and enhanced deacetylation during processing.

As previously mentioned, the selection of the processing route to develop reinforced nanocomposites is sometimes based on the desired properties of the nanocomposites. Therefore, Türkeş & Açıkel (2020) prepared pure halloysite nanotubes and chitosan nanocomposites (MHNT-CTN) for the adsorption of methylene blue via the co-precipitation technique by developing suitable magnetic properties of MHNT-CTN. In another study, the immersion of chitosan-capped ZnO nanoparticles (NCCZO NPs) after a certain period

exhibited good resistance against tubeworms and barnacle formation due to the hydroxyl and amine functional groups (El-saied & Ibrahim, 2020). Hence it can be summarized that the co-precipitation route utilizes a cross-linker to obtain a better morphology, stability, and a physical cross-linking network of molecular chain arrangements with hydrogen bonding. Additionally, the route is also known to be cost-effective and flexible.

Solvent Evaporation Method

Solvent evaporation is a prominent method used for the preparation of biodegradable nanoparticles. According to Gundloori et al. (2019), the technique involves emulsifying polymer in an aqueous phase and dispersion in a volatile solvent such as chloroform, dichloromethane, or ethyl acetate. The authors further stated that the nanoparticles are produced by evaporating the solvent using a high-temperature vacuum or continuous stirring. One of the benefits of this technique is that the parameters, such as particle size, can be tailored by adjusting the evaporation temperature or rate and the stirring rate. In addition, it is perceived that microspheres morphology can be regulated by applying the solvent evaporation method to achieve a targeted purpose.

The properties of bionanocomposites can sometimes depend largely on the evolution of the interactions between the functional groups of polymers and surface hydroxyl groups. Chrissafis et al. (2008) prepared organic/inorganic hybrids by dispersing fumed silica nanocomposites in poly (vinyl pyrrolidone0 (PVP), chitosan, or poly (vinyl alcohol) (PVA) bionanocomposites via solvent evaporation method. This route can prevent the agglomeration of nanoparticles in the prepared bionanocomposites. Denkbas et al. (2004) found that the success rate of the prepared Norfloxacin-loaded chitosan sponges for wound healing purposes was directly proportional to the release rate. It is worth noting that the sponges were able to protect the wound from infections by releasing the loaded antibiotics at predetermined release rates. In the research conducted by El Achaby et al. (2014), it was revealed that the existence of spaces between the sheets and substrates in graphene oxide-reinforced chitosan/polyvinylpyrrolidone polymer improved the strength and toughness of the bionanocomposites, owing to the wrinkled and folded structure of sheets. This modification was accomplished using the solvent evaporation technique, which was chosen to increase the thickness of the graphene oxide sheets.

In a related study, Wu et al. (2011) investigated the interfacial compatibility between multi-walled carbon nanotubes and chitosan nanocomposites (MWCNT/CS) to improve the properties of reinforced chitosan nanocomposites. The authors introduced covalent and ionic linkages into the MWCNT/CS nanocomposites to increase the interaction between the nanocomposites and further initiate chemical interactions to join poly (styrene sulfonic acid) to the surface of the MWCNTs. Due to the nanostructure of chitin nanofillers, which

were used to reinforce chitosan during solvent evaporation, the growth of *A. niger* fungus on the biocomposite films could be impeded (Salaberria et al., 2015). Ifuku et al. (2009) used a similar biocomposite produced through a simple grinding treatment to act as an anti-fungus material. The size and structure of reinforced chitosan biocomposite were modified during processing.

The application of the emulsion solvent evaporation technique has facilitated the fabrication of drug delivery systems with tremendous efficiency. Wang et al. (2017) fabricated biodegradable poly (lactic-co-glycolic acid) (PLGA)-chitosan core-shell nanocomposites as drug carriers. Modifications were done on the nanocomposites by stabilizing the hydrophilic PLGA core with aqueous-phase chitosan to ensure their solubility. This process guaranteed a safe procedure to develop a monodisperse polymer-based drug carrier. To further address the growing interest in a low-cost and effective wound dressing material that has the capability of inhibiting bacterial activities, Santos et al. (2019) developed chitosan and eggshell powder composites with reduced film permeability via solvent evaporation. The authors attributed this significant feature to the presence of remarkable cell viability in the material. To demonstrate the versatility of the solvent evaporation route, Aslam et al. (2021) fabricated copper oxide-reinforced chitosan nanocomposite (CCNC), which was applicable for sensing devices or microelectronics. Analysis results implied a successful fabrication of multifunctional composite films, and the step-by-step procedure is presented in Figure 3. With the solvent evaporation technique, good homogeneous nanocomposites can be produced with improved mechanical properties.

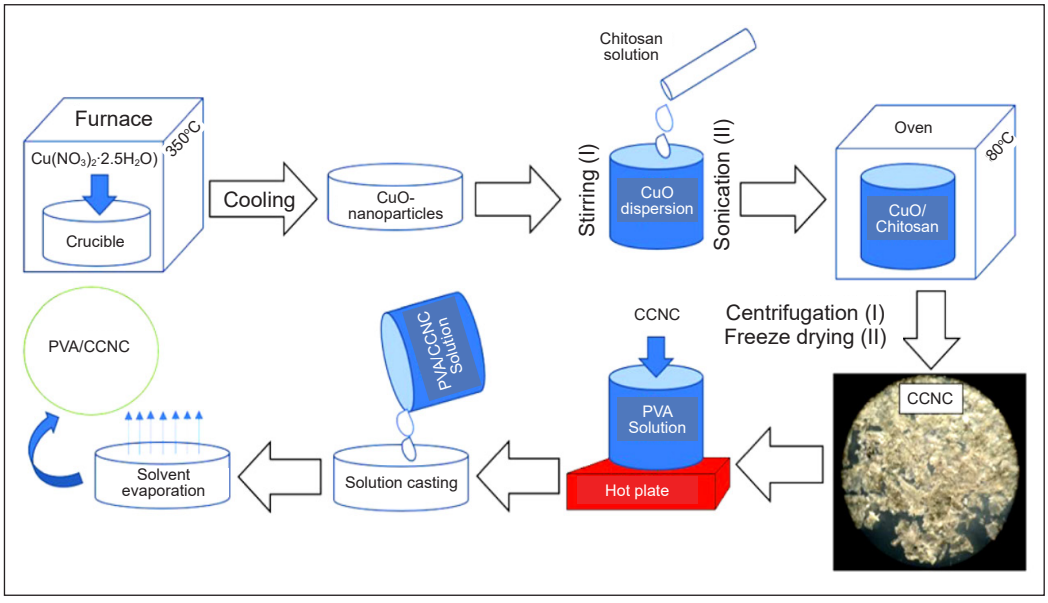


Figure 3. Schematic process of the formation of CuO/chitosan nanocomposites (Aslam et al., 2021)

Sol-Gel Method

Sol-gel is a versatile wet-chemical technique commonly utilized to process ceramic and glassy materials. The procedure is described as a steady evolution of the solution to form a gel-like network, inside which liquid and solid phases are embedded. Two stages must take place to process nanomaterials, namely hydrolysis of precursor in acidic and basic media and poly-condensation of the hydrolyzed mixture. In other words, the sol-gel technique is an approach to modify the surface of the substrates and has the benefit of producing nanocomposites with large surface areas and stable surfaces (Yilmaz & Soylak, 2020).

During processing, the complete dissolution of chitosan and silica in acid and alkaline solutions must be avoided. Therefore, the prepared hybrids should chelate with Cu(II) and Fe(III) ions to a large degree through the sol-gel technique. This method resolved the solubility of chitosan in a weak acid medium while preparing chitosan/silica (CS/Si) via the sol-gel method. Results from the study also indicated the existence of hydrogen bonding within the molecular chain of chitosan and the dispersion of SiO_2, which provide enhanced optical properties to the nanocomposite (Lai et al., 2006). The study by Packirisamy et al. (2019) supported this claim. Furthermore, it was reported that the increase in chelation in ZnO/CS nanocomposite increased the number of dead bacteria and inhibited the growth of *E. Coli*. The schematic diagram of the sol-gel chemical process shown in Figure 4 presents the various ZnO-CS nanocomposites synthesized in the study.

Compared to the blending method, applying the sol-gel method resulted in excellent permeation flux and separation factor in chitosan/titanium oxide composite under similar conditions, owing to the formation of hydrogen and titanoxane bonds within the nanocomposite (Yang et al., 2009). In a related study, to emphasize further the importance of the high surface area in nanocomposites fabricated via the sol-gel technique, Kavitha et al. (2013) reported that a surface area around (114-265 m^2/g) and high purity in titania-chitosan nanocomposite significantly controlled swelling and degradation rate which in turn improved cell growth during implantation and tissue engineering.

In a study by Budnyak et al. (2015), a chitosan-silica composite was synthesized. The composite possessed an excellent morphology due to the high number of available adsorption sites and large surface area, significantly

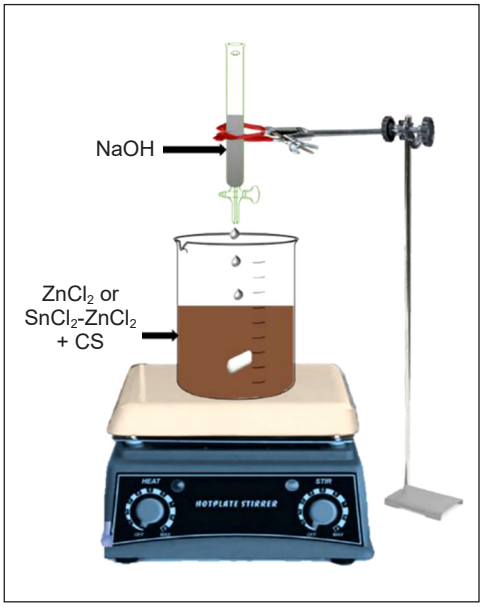


Figure 4. Sol-gel chemical reaction (Packirisamy et al., 2019)

increasing the oxoanions adsorption capacity. It demonstrates the benefit of the sol-gel technique. Furthermore, the sol-gel method could easily be expanded to incorporate organic functionality and hybrid inorganic-organic sol-gels due to its bi-network forming process and the ability to synthesize composite at low temperatures (Budnyak et al., 2016). An excellent homogeneity and cross-link between CS/PEG/E102 and the calcium silicate matrix gave rise to good antibacterial properties of the nanocomposites (Youssef et al., 2017). Owing to a high reaction rate, good biocompatibility, and surface morphology, the prepared chitosan-cl-poly (AA)/$ZrPO_4$ composite exhibited excellent antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* (Sharma et al., 2020). Saravanan et al. (2018) employed the sol-gel coupled with precipitation method to study the crystallinity and bandgap of TiO_2/CS nanocomposite. The analysis performed on the nanocomposite revealed that it possessed good crystallinity and low bandgap, which makes it applicable as an environmental catalyst for textile effluents.

As the sol-gel technique enables the simultaneous synthesis of two or more nanoparticles of different sizes, Rezvani et al. (2018) fabricated Fe_3O4/CS nanocomposites at low temperatures. Improved oil recovery was achieved by virtue of the stable nanocomposites in the suspensions and modified interfacial properties. In another study, a chitosan-doped hybrid/nano-TiO_2 sol-gel coated surface displayed a uniform nanoparticle homogeneity, which resulted in the formation of adherent, crack-free corrosion-resistant coating for aluminum metals (Balaji & Sethuraman, 2017). Hammad et al. (2019) stated that the formation and growth of calcium aluminosilicate (CAS) particles in the chitosan (CS) matrix is a significant factor that increases structural strength in CS/CAS composite doped with aluminum oxide, which can be efficiently utilized as CO_2 gas capturing material at moderate temperatures.

In conclusion, the sol-gel technique is labeled as eco-friendly. Furthermore, it possesses exceptional advantages over other methods as the composition of the nanomaterials can be controlled to achieve excellent homogeneity at the molecular level, and the composite can be produced at low processing temperatures. Table 1 summarizes various processing routes' main highlights and significance in preparing reinforced chitosan nanocomposites and their antibacterial activities.

Electrospinning Method

In recent years, the electrospinning technique has been widely utilized in producing nanofibers. It is a technique that involves the application of electrical voltage to a polymer solution. As a result, the physical and chemical properties of the nanofibers can be modified at the nanoscale level; thus, stronger, more durable, and more elastic fibers can be produced (Schiffman & Schauer, 2008; Meng et al., 2010). The production of nanofibers via the electrospinning process relies on the aggregation of fine fibers from polymer solutions

Table 1
Significance of different processing routes on the morphology and antibacterial activity of chitosan nanocomposites

Composite	Processing route	Key observations	Bacteria specimens	Ref.
Films of chitosan with tetrasulfonated metallophthalocyanines containing (NiTsPc), (CuTsPc) and (FeTsPc)	Electrostatic layer-by-layer (LBL)	• The prepared chitosan films were found to be highly stable and suitable for detecting dopamine concentration • Applicable in bioanalytical fields	—	Siqueira et al. (2006)
Cellulose nanowhiskers and Chitosan	LBL	• Homogeneity in particle distribution and strong interaction between nanocomposites • The processing route is suggested to be applicable in food packaging and biomedical fields	—	de Mesquita et al. (2010)
Cellulose acetate + lysozyme-chitosan-organic rectorite + sodium alginate	LBL	• The use of various deposition conditions influenced the morphology • Improvement in the degree of antibacterial activity	*Escherichia coli* and *Staphylococcus aureus*	Huang et al. (2012c)
Chitosan and tannic deposited on non-fibrous mats	LBL	• An increase in the number of coatings significantly increased antibacterial activity • High antibacterial activity in *S. aureus* due to its sensitivity	*Escherichia coli* and *Staphylococcus aureus*	Huang et al. (2019)
Chitosan + silver oxide nanocomposite	Solution casting	• The processing route can achieve film formation without a cross-linker • The amino group of chitosan interacted with negatively charged microbial cell membranes leading to leakage in proteinaceous and other intracellular constituents of the microorganisms • Bacteria activity was impeded due to the synergistic of $AgNO_3$ and chitosan	*Bacillus subtilis*	Tripathi et al. (2011)
Chitosan poly(vinyl alcohol) (PVA) nanocomposite and sepiolite	Solution casting	• Good dispersion in chitosan matrix and sepiolite • Improved intermolecular hydrogen bonds between chitosan (PVA) and sepiolite	—	Huang et al. (2012a)

Table 1 *(continue)*

Composite	Processing route	Key observations	Bacteria specimens	Ref.
Chitosan and poly(vinyl pyrrolidone)/ nanocellulose (CPN)	Solution casting	• Excellent water vapor and oxygen permeability of CPN maintained a moist environment over the wound bed • The high surface area of nanocellulose aided the adsorption of bacteria microorganisms	*Staphylococcus aureus* and *pseudomonas aeruginosa*	Poonguzhali et al. (2017)
Chitosan + PVA + PVP	Solution casting	• Strong electrostatic forces between chitosan and charged microbial cell membrane inhibited bacteria activity	*Escherichia coli* and *staphylococcus aureus*	Rahmani et al. (2020)
Chitosan + hydroxyapatite (HAp)	Co-precipitation	• Mechanical flexibility and significant homogeneity	—	Yamaguchi et al. (2001)
Fe_3O_4-gold-chitosan	Co-precipitation	• Better morphology and agglomerate-free nanoparticles due to the use of formaldehyde as cross-linker	—	Salehizadeh et al. (2012)
Chitosan + quarternized poly(vinyl alcohol)	Co-precipitation	• Enhanced properties, including conductivity of methanol fuels due to the addition of glutaraldehyde • Enhanced deacetylation	—	Li et al. (2016)
Halloysite nanotubes + chitosan nanocomposites (MHNT-CTN)	Co-precipitation	• Suitable magnetic properties of (MHNT-CTN)	—	Türkeş et al. (2020)
Chitosan nanocomposites + ZnO nanoparticles	Co-precipitation	• The presence of hydroxyl and amine functional groups of (MHNT-CTN) resulted in good resistance to bacteria formation	*Tubeworms* and *barnacles*	El-saied et al. (2020)
Multi-walled carbon nanotubes + chitosan nanocomposites (MWCNT/ CS)	Solvent evaporation	• The solvent evaporation route demonstrated a good interaction between nanoparticles which initiated chemical reactions to improve properties	—	Wu et al. (2011)
Chitin nanofillers + chitosan (CS/ CHNC) and (CS/CHNF)	Solvent evaporation	• The size and shape of chitin nanofillers influenced the inhibition of the activity of the bionanocomposite	A. Niger	Salaberria et al. (2015)

Table 1 *(continue)*

Composite	Processing route	Key observations	Bacteria specimens	Ref.
Poly(lactic-co-glycolic acid) (PLGA) - chitosan	Solvent evaporation	• The solubility of PLGA caused by the aqueous-phase chitosan ensured a safe procedure to develop a monodisperse polymer-based drug carrier		Wang et al. (2017)
Chitosan + eggshell powder	Solvent evaporation	• Reduction in the permeability of the films of chitosan and eggshell powder • The presence of remarkable cellular viability impeded bacteria activities		Santos et al. (2019)
Chitosan + silica	Sol-gel	• Due to excellent hydrogen bonding within the molecular chain of CS and the dispersion of SiO_2, an enhanced optical property of the nanocomposite was achieved		Lai et al. (2006)
ZnO + CS	Sol gel	• Increased chelation of ZnO/CS resulted in the death of bacteria cells and inhibited the growth of *Escherichia coli*	*Escherichia coli*	Packirisamy et al. (2019)
Titania + chitosan	Sol gel	• High surface area and purity controlled swelling and degradation rate, which improved cell growth during implantation	—	Kavitha et al. (2013)
Chitosan polyethylene glycol (PEG) + calcium silicate + modifier (zinc oxide nanoparticles and tartrazine dye)	Sol-gel	• Significant homogeneity and cross-linking yielded good antibacterial activities • Application in optical and biomedical fields • ZnO-NPs are embedded into microbes to interact and bond to the cellular enzyme microbes to inhibit the growth of microbes	*Staphylococcus aureus*, *Pseudomonas aeruginosa* (bacteria), *Candida albicans,* and *Aspergillus niger* (fungi)	Youssef et al. (2017)
Chitosan-cl-poly (AA)/$ZrPO_4$	Sol gel	• The sol-gel route demonstrated a high reaction rate, and good surface morphology resulted in antibacterial activity	*Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Vibrio fischeri*	Sharma et al. (2020)

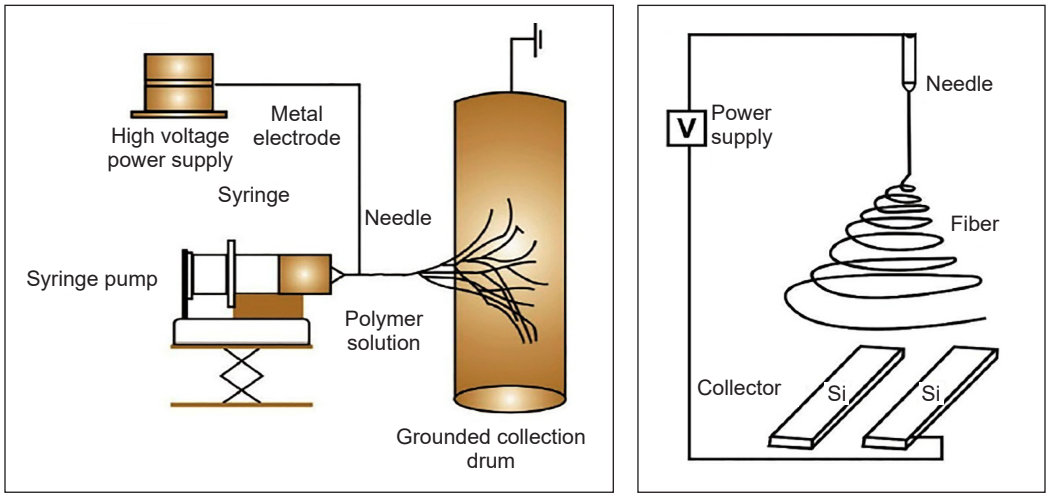
Table 1 *(continue)*

Composite	Processing route	Key observations	Bacteria specimens	Ref.
Chitosan+affinin+AgNP	Electrospinning	• Composite nanofibers showed no defects and exhibited homogenously dispersed orientation • The greater surface area of the nanofiber mats offered significant improvement to the antibacterial activity of the composites	*E. coli* *P. aeruginosa*	Bedolla-Cazares et al. (2016)
Chitosan+PVA+AgNP	Electrospinning	• The fibers exhibited optimum mechanical properties and bactericidal activity		Wang et al. (2018)
Chitosan+Ultra-high molecular weight polyethylene oxide	Electrospinning	• Improved benefits in terms of antibacterial and osseo-conductive properties. • Lesser swelling upon immersion in water.		Qasim et al.(2018)
Chitosan+PVA	Electrospinning	• Improved antibacterial activity against *S. aureus* and *P. aeruginosa*	*S. aureus* *P. aeruginosa*	Abbaspour et al. (2015)
Chitosan+Polyethylene oxide+AgNP	Electrospinning	• Improved antibacterial activity against *E. coli and S. aureus*	*E. coli* *S. aureus*	Wang et al. (2015)

through electrostatic forces. The critical components of an electrospinning setup are (i) a high-voltage power supply, (ii) a spinneret and a grounded collecting plate, and (iii) a plate or rotating mandrel (Figure 5) (Zafar et al., 2016).

The electrospinning process of chitosan/ultra-high molecular weight polyethylene oxide (CH-UHMWPEO) solutions was reported by Qasim et al. (2018). The major advantages of the produced fibers were the high chitosan and low PEO contents, which led to lesser swelling upon immersion in water. In addition, increasing the CH content improved the antibacterial and osseo-conductive properties. A great deal of research was carried out to enhance the stability, regenerative property, and antimicrobial property against *Staphylococcus aureus* and *Escherichia coli* of electrospun CH-PEO fibers combined with poly(hexamethylene biguanide) hydrochloride (PHMB) or silver nitrate nanoparticles (Dilamian et al., 2013; Penchev et al., 2010). These fiber-based scaffolds exhibited promising potential as wound dressings to prevent infections and accelerate healing.

In another study, nano-silver particles added to electrospun CH/PEO fibers demonstrated antibacterial activity against *S. aureus* and *E. coli*, which are the dominant organisms that cause wound infections (Wang et al., 2015). Similarly, electrospun chitosan/polyvinyl alcohol (CH-PVA) fibers incorporated with mafenide acetate exhibited antibacterial activity against *S. aureus* and *P. aeruginosa* (Abbaspour et al., 2015). Elsewhere, fibers with silver nanoparticles were produced through the in-situ reduction of $AgNO_3$ into a chitosan-polyvinyl alcohol mixture (CS/PVA: 1/10, w/w), which was dissolved in lactic acid (Wang et al., 2018). It was observed in the study that the optimum mechanical properties and bactericidal activity were exhibited by the fibers with the least content of nanoparticles, which complied with the high surface-to-volume ratio resulting from their low dimension.



(a) (b)

Figure 5. (a) Electrospinning equipment plate or rotating mandrel, and (b) aligned collection plates for electrospun nanofibers (Zafar et al., 2016)

In a study by Dobrovolskaya et al. (2018), composite nanofibers based on chitosan and chitin nanofibrils were prepared. The fibers were spun from an aqueous solution containing chitin nanofibrils and underwent ultrasound treatment for 40 minutes. The nanoparticle concentrations were in the range of 1 to 30 wt% with respect to chitosan. The electrospinning of the fibers was performed by employing the NANON-01A setup. The solution was fed into the setup by an injection pump via a spinneret electrode operating in an electric field at a voltage of 18 kV. A distance of 0.2 m was set between the spinneret electrode and the collector electrode, where the fibers were deposited. The effectiveness of the electrospinning technique in producing chitosan nanocomposites with silver nanoparticles from the $AgNO_3$-affinin complex was studied by Bedolla-Cazares et al. (2017) (Figure 6). The chitosan/AgNP composite solution, prepared via chemical reduction, was dried at 74°C for eight hours to evaporate the solvent. The resulting films were re-dissolved in a TFA-CH2Cl2 mixture to produce 12 wt% solutions, which were later processed in a NaBond® electrospinning unit to produce nanofiber mats.

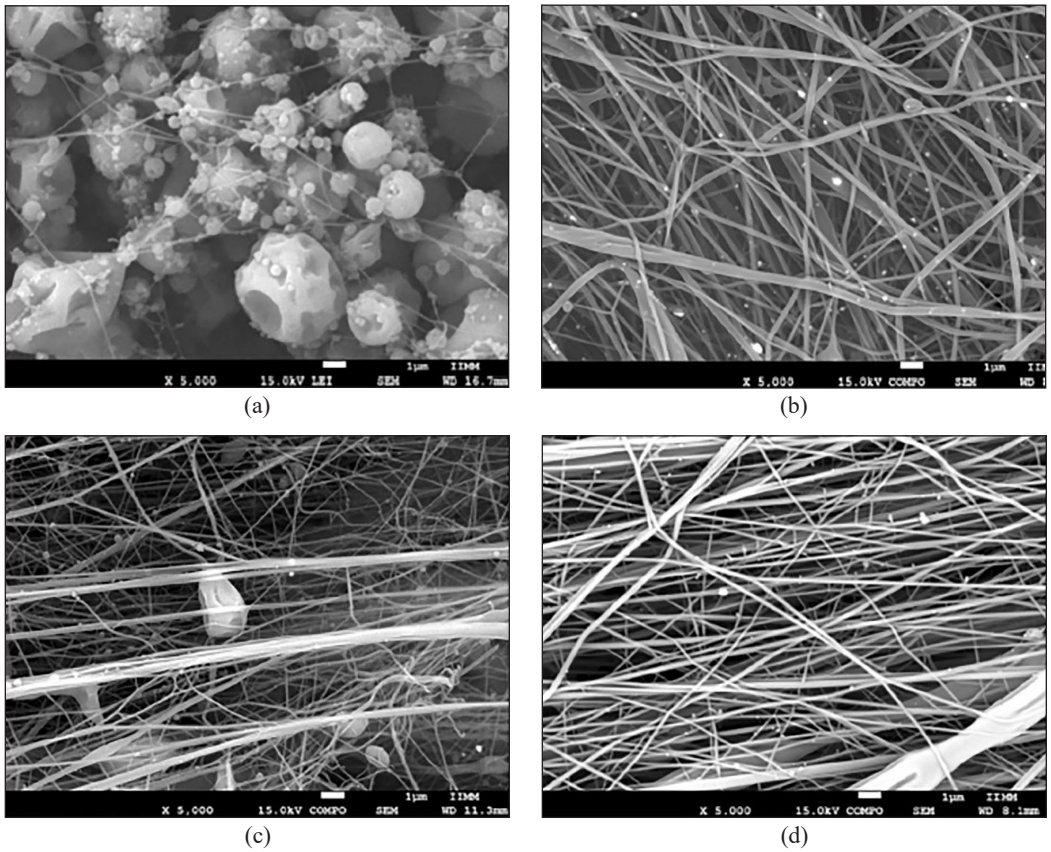


Figure 6. Electrospun nanofibers from (a) Chitosan/affinin, (b) Chitosan/$AgNO_3$ (after reduction), (c) Chitosan/affinin + $AgNO_3$ added independently (after reduction), (d) Chitosan/$[Ag_2$-(affinin)$](NO_3)_2$ complex (after reduction) (Bedolla-Cazares et al., 2017)

In work by Haider and Park (2009), as-spun nanofibers were neutralized using potassium carbonate to prevent fibrous structure loss when the fibers come into contact with neutral or weak basic aqueous solutions (Haider & Park, 2009). As seen in Figure 6(c), the introduction of $AgNO_3$ into the chitosan/affinin mixture promoted the production of nanofibers. Nevertheless, large agglomerates were still evident. This drawback was overcome when the affinin was complexed with $AgNO_3$. Figure 6(d) shows that the CTS/affinin/AgNP composite nanofibers were successfully formed without defects and with a more homogenously dispersed orientation. The antibacterial activity test results show that *E. coli* and *P. aeruginosa* exhibited limited activity in the inhibition zones of the thin composite films produced with affinin and AgNP. Due to the greater surface area of the nanofiber mats, the antibacterial activity of pure chitosan and composite combinations was significantly enhanced.

MECHANICAL PROPERTIES OF CHITOSAN NANOCOMPOSITES

Chitosan is a polysaccharide obtained from the exoskeletons of arthropods, crustaceans, insect cuticles, plants, and the cell wall of fungi. On the one hand, the natural origin of chitosan endows it with good hemostatic, antibacterial, and non-toxic properties, in addition to biocompatibility, biodegradability, and bio-inertness. These properties make it suitable for composite materials in wound healing and other biological applications which involve direct contact with physiological fluids. On the other hand, poor mechanical properties of unreinforced chitosan constitute a major setback, especially in structural and load-bearing applications such as tissue engineering. It is because of the destruction of the chitin multiscale fibrillar structure during its conversion to chitosan, which affects further processing and exploitation. Chitosan is often combined with other materials to form bio-based blends with improved properties to overcome this setback. Thereby, their advantageous innate properties could still be exploited. This section highlights the mechanical properties of various chitosan blends.

Tensile Properties

Generally, pure chitosan film has low tensile strength due to its low crystallinity (Li et al., 2021). Therefore, combining chitosan with other materials, such as polylactic acid (PLA), is often beneficial. For example, Elsawy et al. (2016) studied the mechanical properties of PLA nanocomposites prepared by reinforcing PLA with chitosan nanoparticles (CSNP) at a proportion between 0 and 5%. The presence of chitosan nanoparticles in the nanocomposites produced progressive improvement in tensile strength. It was also reported that reinforcing PLA with chitosan nanoparticles altered the tensile strength at the breaking point, which increased progressively with an increase in the proportion of the reinforcement. Based

on these findings, it is possible to further improve the tensile strength of pure PLA by increasing the proportion of chitosan.

In related research, Boonkong et al. (2013) investigated the suitability of deacetylated chitosan blended with various proportions of polylactic acid (PLA) and polycaprolactone (PCL) as wound dressings. As Khan et al. (2000) established, good wound dressing devices must be flexible yet stiff enough to withstand blood pressure from wounds. The chitosan-PLA-PCL blends possessed these features. PLA enhanced the strength, while PCL increased the flexibility and elasticity of the biocomposite. The mechanical properties of the blend, namely tensile strength, young's modulus, and percentage elongation, were studied using Universal Testing Machine (UTM) according to the ASTM D 882 standard. The temperature and humidity were 25°C and 50%, respectively, whereas the crosshead speed and load cell were set at 12.5 mm/min and 5.0kN, respectively. The study revealed that 60 wt% chitosan, 28 wt% PLA, and 12 wt% PC produced a blend with improved tensile and adhesive strengths and superior swelling and water vapor transmission rate. It was assumed that the improved mechanical properties could enhance the surface interaction between the film and wound tissues to promote a faster healing process, especially when the film was loaded with drugs for controlled delivery. This assumption was confirmed by performing a blood clotting test on the blend (60% chitosan, 28% PLA, and 12% PCL) loaded with doxycycline (0.03 g) and monosodium glutamate MSG (0.1 g). The test results revealed an outstanding blood clotting capability of the biocomposite. Moreover, the in vitro study of this film blend showed that it could prevent hemolysis and bacterial infection.

Aside from synthetic materials such as PLA, blending chitosan with other materials of natural origin, which possess properties that can improve the mechanical properties of the blend, such as tensile and compressive strength, can be beneficial. One such material is natural silk, which possesses high crystallinity and good mechanical properties. However, it is also regarded as one of the toughest natural materials (Li et al., 2021; Zhang et al., 2015). The complex hierarchical structure of silk, which includes high molecular weight, β-sheet crystal, and fibrillar structures, is the underlying reason for its excellent mechanical properties, making it a good reinforcement candidate for chitosan (Keten et al., 2010; Vollrath & Knight, 2001).

In a study by Li et al. (2021), silk nanofibrils solution (SNF), which was obtained from silkworms, was combined with chitosan (CS) solution based on the weight percentage to obtain various blends of SNF-CS biocomposites, which mechanical properties were tested. The study revealed that reinforcing chitosan films with 25 to 75 wt% SNF significantly improved their tensile strength from 29.3 to 40.1 MPa (Figure 7). Nevertheless, a decrease in the compressive strength of the composite films was observed when the weight percentage of SNF was further increased. It suggests that a high proportion of nanofiber in the nanocomposite may weaken the interfacial binding force, leading to a reduction in the

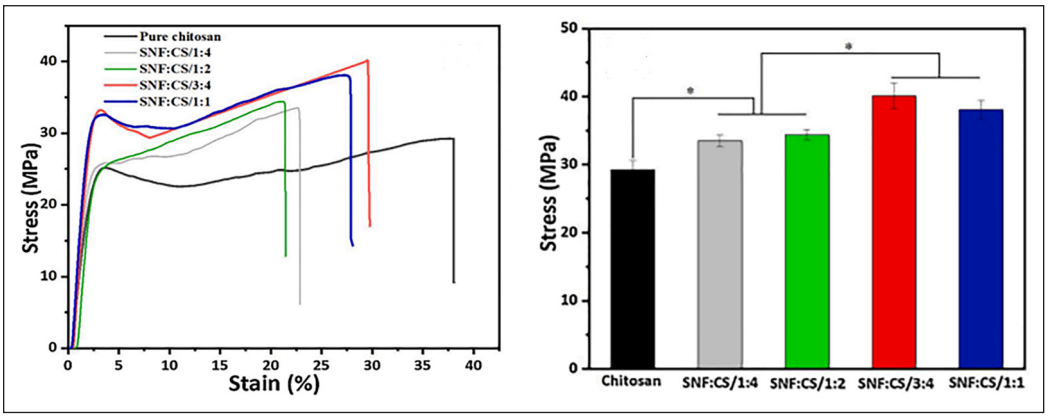


Figure 7. Stress-strain curve of Silk nanofibril-Chitosan nanocomposite (Li et al., 2021)

mechanical properties of the matrix phase, which in turn inhibits further improvement in the tensile properties. In addition, a decrease in elongation at fracture was observed after the SNF loading was increased. It indicates that decreasing the SNF loading increases the brittleness of the chitosan nanocomposites.

Like other bio-based materials, chitosan processing methods could influence its mechanical properties and wound healing capabilities. It was confirmed in a study in which chitosan films were prepared by dissolving them in two solvents: lactic acid (LA) and acetic acid (AA). The mechanical properties of Chitosan-AA and Chitosan-LA biofilms were compared with those of a commercial Omiderm film. Both films exhibited superior mechanical and bioadhesive properties as compared to Omiderm film. More importantly, compared to Chitosan-AA film, Chitosan-LA film possessed lower tensile strength with longer elongation at fracture, which endowed it with better flexibility, remarkable softness, and superior pliable properties. As a result, Chitosan-LA was more bioadhesive and hence, more applicable to wound healing and skin burn management than Omiderm and Chitosan-AA films. In addition, due to its flexibility, Chitosan-LA film had good permeability to water vapor, was non-irritant, and did not induce any adverse reaction on the skin. These are the expected characteristics of a typical wound management construct (Khan et al., 2000).

Compressive Properties

Biocomposites are a blend of various materials, and their compressive properties are expected to differ from one blend to another, depending on the materials involved. Research has shown that chitosan forms electrostatic conjugates with other negatively charged polymers (natural or synthetic) because it is either neutral or negatively charged in an acidic medium. Therefore, it has led researchers to conjugate chitosan with various polymers. One such research involved reinforcing PLA (95% L-lactide and 5% meso-lactide) dried at 50-60°C under vacuum using an electric air convection heater, with varying

proportions of chitosan powder particles ≤25μm. The compressive test results revealed that the compressive strength of the biocomposite could be increased by increasing the proportion of chitosan reinforcement (Singh et al., 2020).

Developing porous scaffolds from biomaterials has been the main focus in wound healing and tissue engineering applications, especially biomaterials of natural origins, such as silk nanofibrils-Chitosan (SNF-CS) biocomposite (Wang et al., 2013). In weight-bearing applications such as meniscal tissue regeneration, the 3D scaffolds are mostly subjected to compression. Therefore, the need to study the mechanical properties of porous scaffolds under compression is even greater. Several researchers have suggested that the presence of biopolymer nanofibrils such as chitin nanocrystals in nanocomposites increases the compressive strength and the modulus of chitosan scaffolds (Dresvyanina et al., 2020; Yin et al., 2019; Liu et al., 2016). Furthermore, tissue scaffolds mimic the natural extracellular matrix of the biological system, which is usually in wet conditions due to the contact with physiological fluids, for instance, in wound healing applications. Several biochemical processes and cellular activities occur in this moist environment, including cell migration, signaling, differentiation, growth, survival, and other conditions that keep the extracellular matrix in good health. Because of this, it is necessary to examine the behavior of tissue scaffolds in wet and dry conditions to give an idea of their suitability in various in vivo conditions.

In work involving silk nanofibril-chitosan porous scaffolds, Li et al. (2021) reported significant improvement in the mechanical properties of SNF/CS nanocomposites scaffold, with 75% of the SNF/CF scaffold in the wet state as compared to pure chitosan scaffolds. Furthermore, the modulus of elasticity in compression and the strength at 0.6 strain had improved remarkably, indicating that the chitosan scaffolds can be successfully reinforced with SNF for use under wet conditions. It verifies that the porous scaffolds' mechanical properties, especially the compressive properties, are strongly affected by the nanofiber ratio and the solid content.

Impact Strength and Nanoindentation

An investigation of the impact strength of biocomposite material could provide insight into its ability to retain its structural integrity after being loaded by a suddenly applied force. It usually entails mounting a test specimen tightly and hitting it with a pendulum at a known impact force. For example, Elsawy et al. (2016) conducted an Izod impact test on PLA reinforced with chitosan nanoparticles. The presence of 1 and 3% chitosan nanoparticles in the PLA-CSNP composite significantly enhanced its impact strength. However, increasing the chitosan reinforcement to 5% decreased the impact strength of the biocomposite. It suggests that PLA should be moderately reinforced with chitosan nanoparticles to produce composites suitable for applications requiring an impact strength higher than pure PLA.

Wang et al. (2005) used the continuous stiffness measurement (CSM) technique with a tri-sided pyramidal diamond indenter to examine the mechanical behavior of biopolymer chitosan-montmorillonite nanocomposites at the nanoscale level. It involved pressing the nanoindenter into a 60 μm thick chitosan test specimen and clay nanocomposites film at a strain rate of 0.05 1/s up to a depth of 3000 nm. The authors reported that clay-reinforced nanocomposites had higher moduli than non-reinforced composites. Furthermore, it was observed that increasing the clay concentration increased the modulus profile and stiffness of the nanocomposites. In addition, reinforced nanocomposites exhibited a higher hardness profile than their unreinforced counterparts. Hence, it can be concluded that the hardness and elastic modulus of chitosan nanocomposites can be enhanced by increasing the clay concentration.

Similarly, Zhu et al. (2011) utilized the atomic force microscopy nanoindentation process to investigate the hardness and modulus of soft tissue scaffolds made from chitosan. It involved pressing spherical indenters with diameters of 5 and 10 μm for investigating nano hardness and modulus of indentation, respectively, on two chitosan nanofilms produced by reinforcing cross-linked and non-cross-linked chitosan with graphene and fullerene of varying proportions. They reported that improved hardness was the result of increasing the nanofillers proportion. The mechanical properties of various blends of materials with chitosan are summarized in Table 2.

Table 2
Mechanical properties of different chitosan nanocomposites

Composite	Mechanical Test	Key observations	Ref.
Chitosan film-silk nanofibrils	Tensile	Silk nanofibrils reinforcement improved the tensile strength of the chitosan film up to 40.1 MPa.	Li et al. (2021)
Chitosan-polylactic acid	Tensile	Improved tensile strain (ductility) with increasing reinforcement from 1-3%.	Elsawy et al. (2016)
Chitosan-polylactic acid-polycaprolactone	Tensile	Superior tensile and adhesive strengths were exhibited at 60 % chitosan, 28 % polylactic acid, and 12 % polycaprolactone	Boonkong et al. (2013)
Chitosan film-silk nanofibrils	Compressive	Improved compressive modulus of elasticity and strength at 0.6 strain	Li et al. (2021)
Chitosan-polylactic acid	Compressive	Enhanced compressive strength with rising content of reinforcement.	Singh et al. (2020)
Chitosan-polylactic acid	Impact	Improved impact strength was achieved by up to 3 % of reinforcement addition.	Elsawy et al. (2016)
Chitosan-montmorillonite	Nanoindentation	Reinforced chitosan film exhibited a higher hardness profile relative to the unreinforced counterpart.	Wang et al. (2005)
Chitosan scaffold	Nanoindentation	Improved hardness with increasing addition of nanofillers	Zhu et al. (2011)

CONCLUSION

This paper provides a comprehensive insight into the currently known processing techniques of chitosan nanocomposites and highlights their potential as viable wound healing material. The performance of chitosan nanocomposites is largely related to the homogenous distribution of fillers within their polymeric matrix. The successful distribution creates an excellent chitosan/filler interface, which often creates a high specific interfacial area required to achieve the full potential of the developed nanocomposites. Among the processing techniques reviewed in this paper, the LBL is the most reliable technique for drug delivery applications. The particles are homogeneously distributed and could interact effectively to ensure contact between the bacteria and LBL film. Through the solution casting method, fillers such as MWCNTs and HTs can be homogeneously dispersed in the chitosan, resulting in enhanced mechanical properties of the composites. The antibacterial effects can be achieved with the introduction of AgNPs or ZnO. Sol-gel is notably environmentally friendly, and the nanomaterial composition can be controlled to achieve remarkable homogeneity. Future studies are crucial to give insights into various cost-effective processing techniques. A comprehensive characterization of chitosan nanocomposites can ascertain their conformity to existing standards in the clinical setting.

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