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Chitin nanofibers: recent advances in preparation and applications in biomedical and beyond

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Chitin and chitosan-based nanofibers (ChNFs), derived from renewable sources, have emerged as promising biomaterials due to their unique properties such as high surface area, porosity, biocompatibility, and biodegradability. This review provides a comprehensive overview of ChNF extraction and synthesis, focusing on both top-down and bottom-up approaches. A comparative analysis of these methods is presented, highlighting the challenges, opportunities, environmental impact, cost-effectiveness, and quality consistency associated with each. The advantages of ChNFs over similar nanomaterials are elucidated, emphasizing their diverse applications in biomedical and environmental fields. Biomedical applications include drug delivery, tissue engineering, cancer treatment, wound healing, and biosensing. Environmental applications encompass water treatment, air filtration, agriculture, and biodegradable packaging. Despite their potential, challenges remain, including low solubility, unstable mechanical properties, and inconsistent quality, which limit their widespread use. This review also examines recent advancements in ChNF research, aiming to guide the development of efficient and environmentally friendly synthesis methods. By encouraging innovation in ChNF-based nanotechnologies, this research contributes to a more sustainable future.

1 Introduction

Chitin and its deacetylated derivative, chitosan, are renowned cellulose analogs characterized by a repeating (1,4)-*N*-acetyl glucosamine structure.[1] As the second most abundant biopolymer after cellulose,[2,3] chitin is predominantly found in the exoskeletons of crustaceans and the cell walls of fungi.[4] Despite being biosynthesized at a rate of 10^{10} to 10^{11} tons annually, most chitin is discarded as waste.[5] Therefore, the efficient utilization of chitin as a sustainable green material is paramount. Chitin's linear structure, featuring two hydroxyl groups and an acetamide group, contributes to its high crystallinity, strong hydrogen bonding, and organization into antiparallel nano-sized chitin nanofibers (ChNFs) (Fig. 1).[6] The ChNFs, typically 2–5 nm in diameter and 300 nm in length, are embedded within a protein matrix.[7–10] The hierarchical ChNF-based structure of crab and prawn shells suggests that the isolation methods employed for cellulose nanofibers could be applied to other chitin-containing biomass sources.[10]

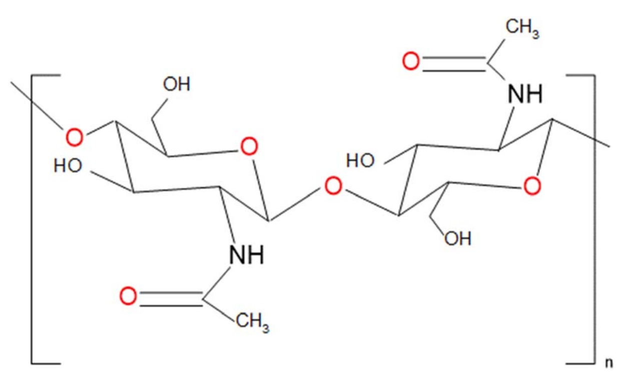

Fig. 1 Structure of the chitin molecule, showing two *N*-acetylglucosamine units that repeat to form long chains in the β-(1 → 4)-linkage.

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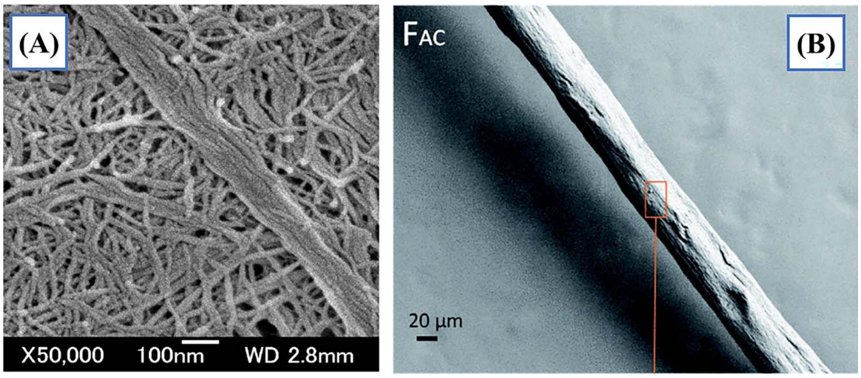

Fig. 2 (a) Nanofibers[5] and (b) micro-sized fibers.[21]

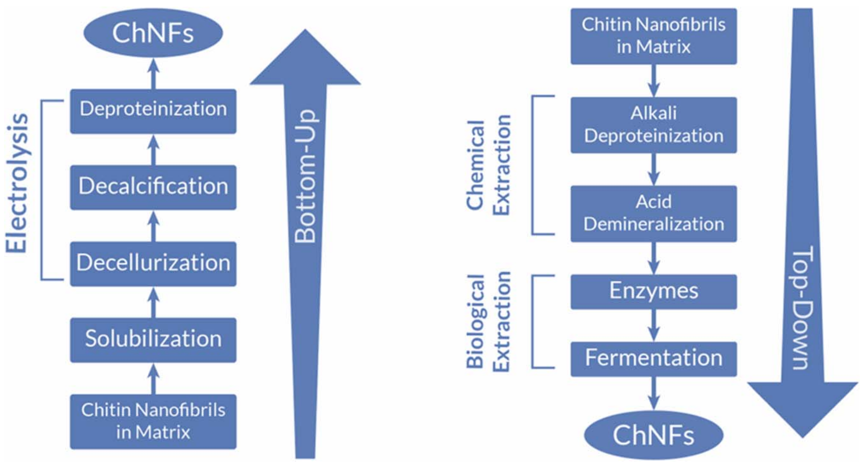

Fig. 3 Extraction of ChNFs *via* different routes of 'bottom-up & top-down' approach (**recreated from the text and other informations).

ChNFs, defined as fibers with diameters below 100 nm and aspect ratios exceeding 100 [6,11] are fundamental building blocks in natural biological materials. Their assembly occurs in various biopolymers, including polypeptides like silk fibroin[12,13] collagen,[14] keratin,[15] and polysaccharides like cellulose and chitin.[16] The unique properties of ChNFs, distinct from those of micro-sized fibers, arise from their exceptionally high surface-to-volume ratio[17] and the formation of highly porous meshes (Fig. 2). Due to their distinctive dimensional, optical,[18] mechanical,[19] and other properties, the preparation of ChNFs is a critical endeavor. While electrospinning is a common artificial method for producing ChNFs from polymer solutions,[20] it has a significant environmental impact. Consequently, there has been growing interest in deriving ChNFs from biopolymers due to their environmentally friendly attributes, such as renewability, biocompatibility, biodegradability, and sustainability.[1]

Nature produces a diverse array of ChNFs, including collagen triple helix fibers, keratin fibrils, and fibroin fibrils. Natural ChNFs are extracted by downsizing the structures of biomass-derived organizations, a process considered a "top-down" approach.[11] In contrast, electrospinning is deemed a "bottom-up" approach, as it involves bundling molecules into ChNFs.

The ChNFs can be prepared through top-down and bottom-up processes (Fig. 3).[11] However, compared to the abundant research on the bottom-up preparation of homologous NFs from chitin and cellulose, there have been fewer reports on the top-down production of ChNFs. Acidolysis of amorphous domains in semicrystalline chitin has resulted in the formation of chitin nanocrystals,[22] which are suitable for reinforcing polymer nanocomposites. However, their low aspect ratio does not align with the natural fibril form of chitin found in crabs and prawns.

2 Strategies for preparation of ChNFs from chitin

2.1 Top-down

The ChNFs hold significant promise in various applications due to their unique properties. However, the inherent insolubility of chitin necessitates a top-down approach for their production, primarily utilizing crustacean shells as the starting material. This approach involves breaking down bulk chitin into its nanoscale building blocks.

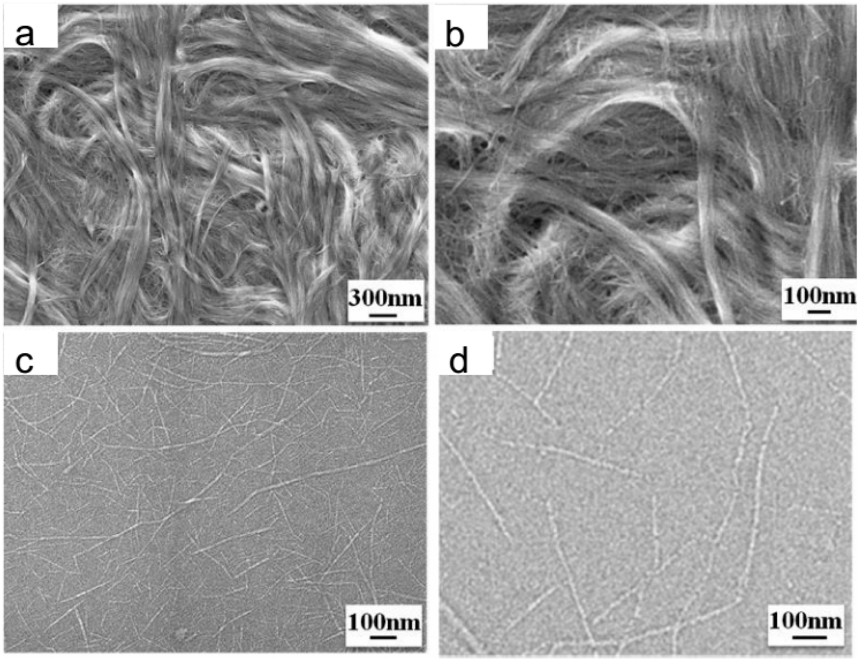

Fig. 4 (a and c) SEM images of the cationized chitin and homogenized chitin with a pH of 4.1, respectively; (b and d) SEM images with higher magnification in comparison to (a) and (c), respectively.[30]

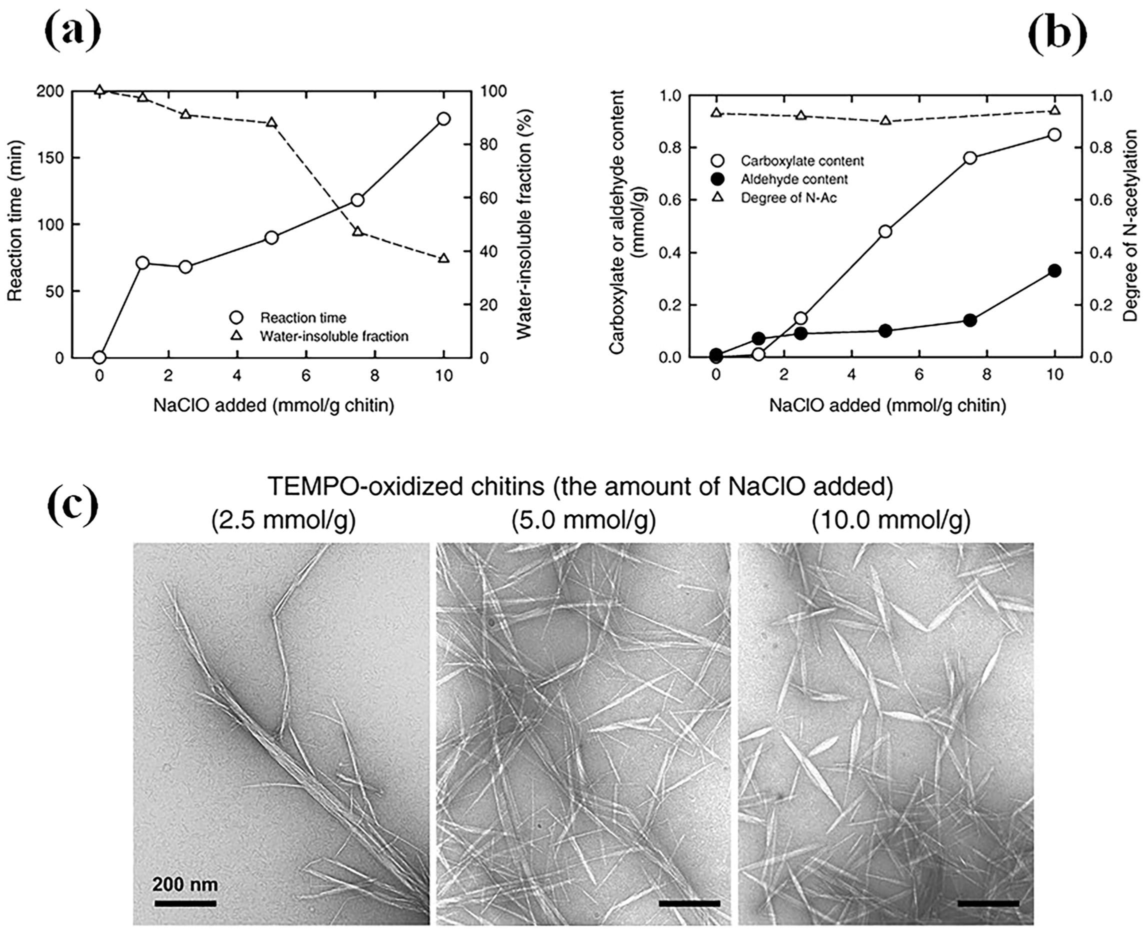

Fig. 5 (a) Relationships between the amount of NaClO added in the TEMPO-mediated oxidation of chitin and either the total reaction time or the weight ratio of the water-insoluble fraction. (b) Relationships between the amount of NaClO added in the TEMPO-mediated oxidation of chitin and either the carboxylate or aldehyde content of the water-insoluble fraction. The degree of *N*-acetylation is also plotted. (c) TEM images of TEMPO-oxidized chitin nanocrystals prepared under different conditions.[29]

The initial step typically involves the purification of chitin from crustacean shells through demineralization and deproteinization using acid and alkali treatments.[23–25] Subsequently, the purified chitin undergoes acid hydrolysis, which cleaves the non-crystalline regions. The acidic environment protonates the primary amine groups, leading to a more stable colloidal suspension with smaller nanofibers.[25,26]

Goodrich *et al.*[27,28] developed a method involving high-speed blending of a neutralized chitin suspension followed by lyophilization to produce ChNFs. However, this method often results in nanofibers larger than their naturally occurring counterparts (~3 nm) and with a broad diameter distribution. Alternatively, grinding the chitin suspension at neutral pH yields smaller nanofibers (10–20 nm). This approach, however, requires the chitin to be directly extracted from crustacean shells and maintained in a hydrated state to prevent strong hydrogen bonding between fiber bundles upon drying.[5,28]

High-pressure homogenization offers a milder alternative. In this method, a pristine chitin dispersion at pH 4.1 is passed multiple times through a high-pressure homogenizer, resulting in a chitin/water dispersion that can be cast into thin films with an average nanofiber diameter of 20 nm [29] (Fig. 4).

Another top-down approach involves the mediated oxidation of chitin. This method utilizes 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) along with sodium hypochlorite (NaClO) as a co-oxidant to selectively oxidize the primary hydroxyl groups of chitin to carboxylate groups.[30–32] By controlling the NaClO concentration, the extent of oxidation can be adjusted, influencing the water-insoluble content while preserving the degree of *N*-acetylation (Fig. 5).[29] The negatively charged carboxylate groups on the chitin crystallite surfaces facilitate the breakdown into ChNFs.

Partial deacetylation of chitin to chitosan, followed by protonation of the resulting primary amines, is another strategy for ChNF production. This method exploits electrostatic repulsion to achieve individual nanofiber separation. Pristine crab shell chitin is treated with NaOH at elevated temperatures to increase the number of primary amines.[33] Subsequent mechanical disintegration yields individual ChNFs with diameters of approximately 6 nm and higher aspect ratios compared to TEMPO-mediated ChNFs.[34]

Oh *et al.*[35] reported a environmentally friendly method for disintegrating chitin using calcium ions and solvent exchange. This innovative approach produces a hierarchical chiral nematic phase, mimicking the Bouligand structure found in nature (Fig. 6). Chitin is dissolved in a Ca-saturated methanol solution, where Ca^{2+} ions disrupt intramolecular hydrogen bonding. Solvent exchange with methanol, isopropanol, and deionized water then removes the Ca^{2+} ions, yielding ChNFs that exhibit nematic or liquid crystalline phases in alcohol or chiral nematic phases in hydrogels.

The various top-down approaches for ChNF synthesis are illustrated in a flowchart (Fig. 9).[36]

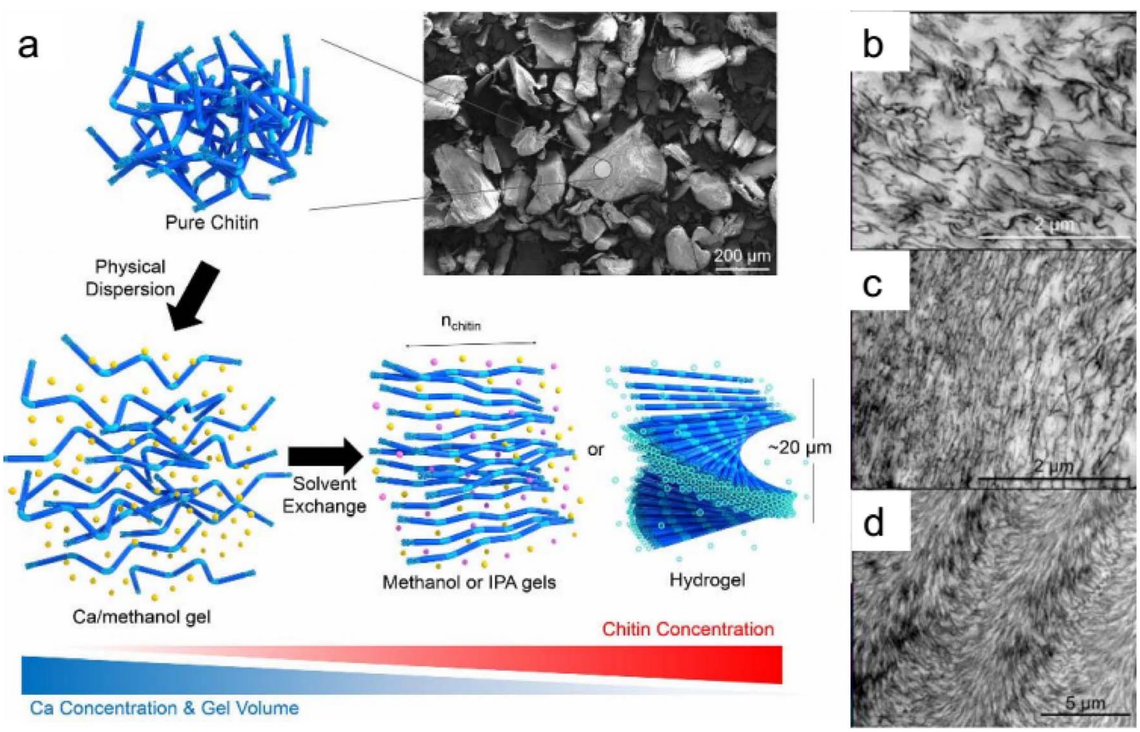

Fig. 6 (a) Calcium-saturated methanol disintegrates ChNFs with minimal chemical modification, generating a Ca-methanol gel (disordered) (bottom-left panel). Ca^{2+} is removed from the Ca-methanol gel by washing with alcohol (methanol or IPA) and DI water, thus generating alcohol gels (methanol gel or IPA gel) in the N phase (bottom-middle panel) and a hydrogel in the N* phase (bottom-right panel). The yellow, pink, and blue beads represent three types of solvent molecules: methanol-solvated Ca^{2+}, alcohol (methanol or IPA), and water. (b) TEM images show a morphological change in chitin nanowires by solvent exchange of Ca-methanol gel, (c) IPA gel, and (d) hydrogel.[35]

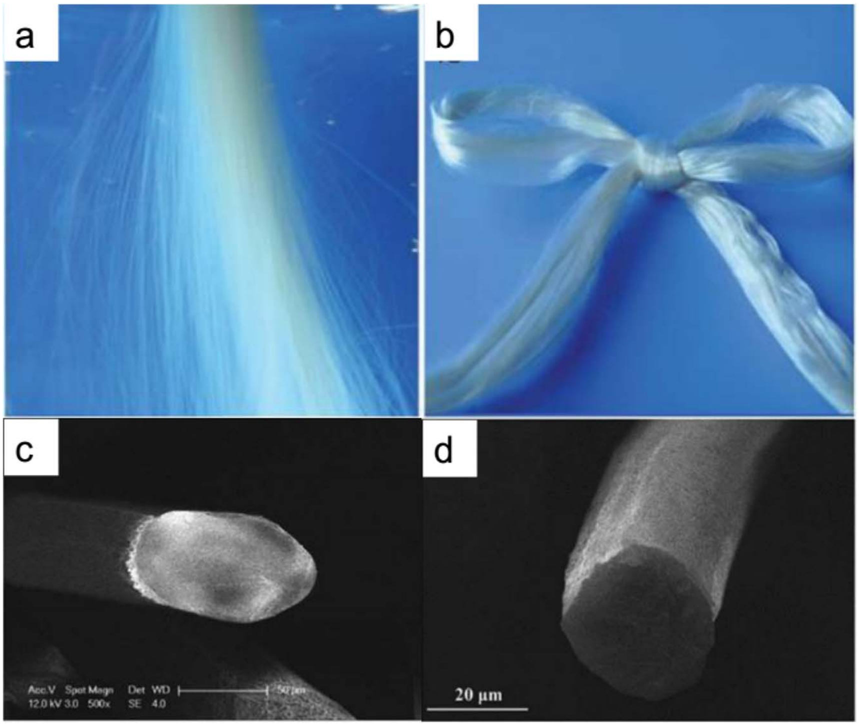

Fig. 7 Photograph of (a) freshly spun chitin fibers in water and (b) air-dried fibers. SEM images of the chitin fibers (c) lyophilized and fractured in liquid nitrogen and (d) air-dried.[44]

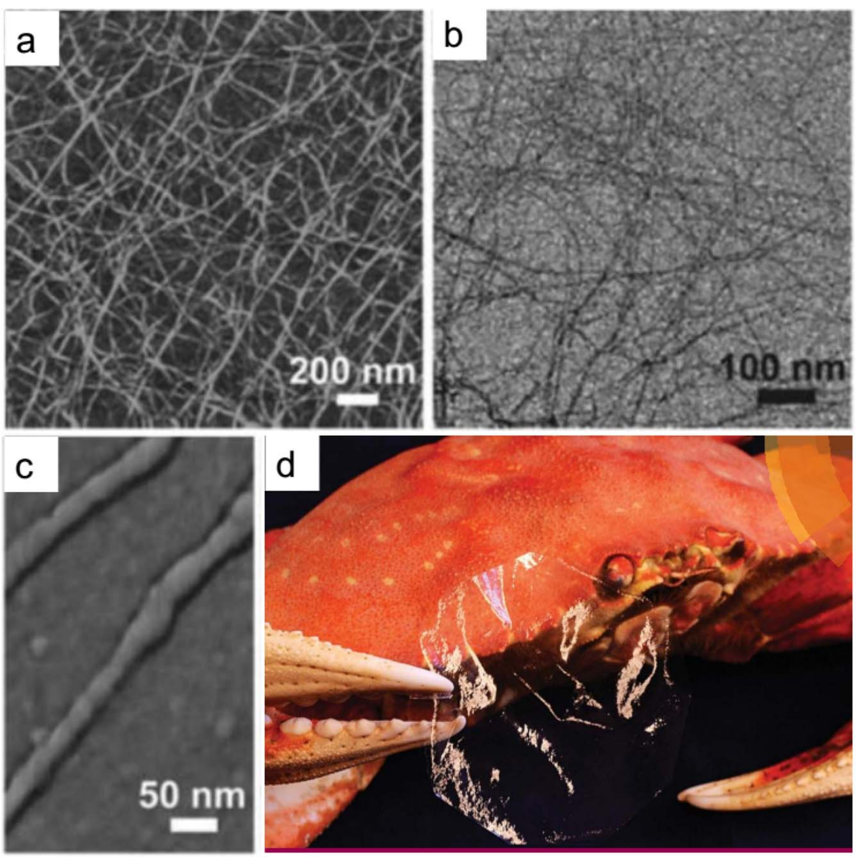

Fig. 8 The morphology and diameter distribution of ChNFs. In the AFM images, the apparent nanofiber width is larger due to tip convolution. (a–c) 3 nm nanofibers prepared from HFIP solution (5 mL, 0.01 wt%): (a) AFM height image, (b) bright field TEM image, (c) AFM phase image of two fibers. (d) Thin transparent chitin film fabricated from HFIP solution drop-casting[45] (a–c) and[47] (d).

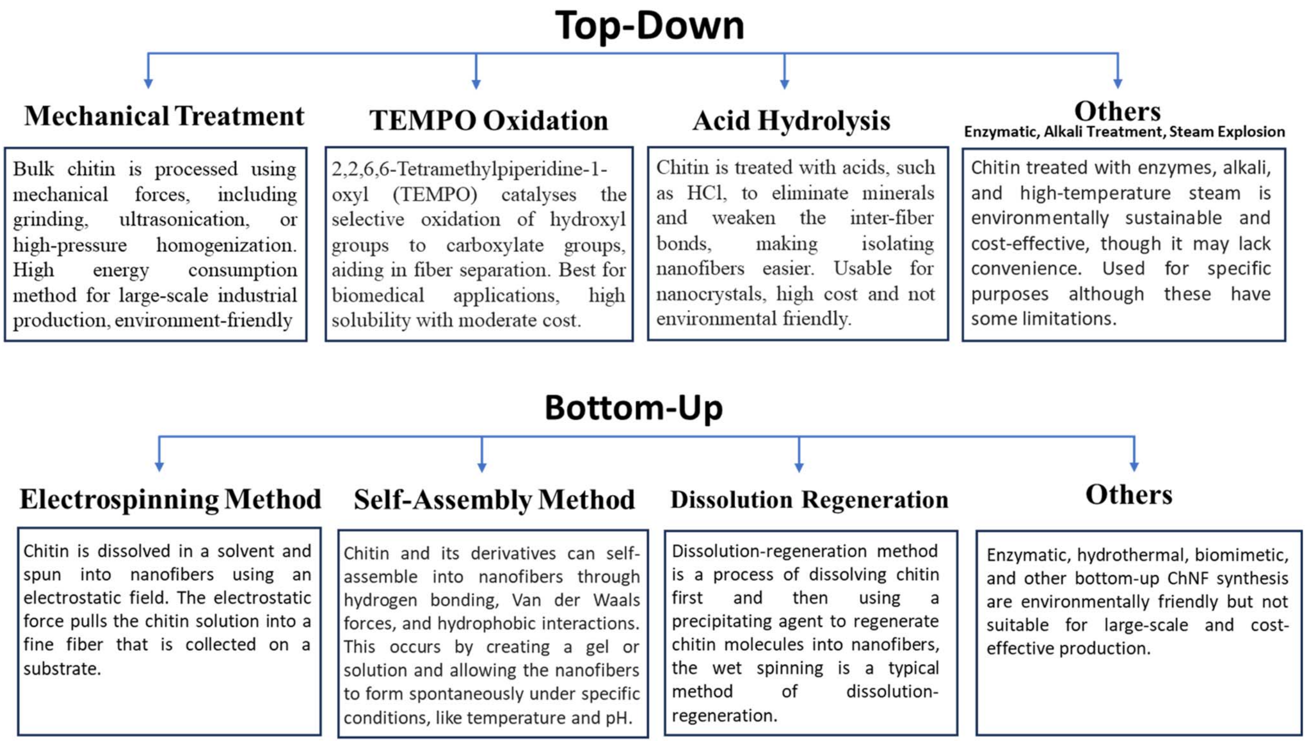

Fig. 9 The various top-down and bottom-up approaches for ChNF synthesis.[36]

2.2 Bottom-up

In contrast to the top-down approach, the bottom-up approach to ChNF production involves assembling nanofibers from individual molecules into organized structures. This approach requires the dissolution of chitin molecules, posing a challenge due to chitin's limited solubility in water and most organic solvents.[37]

Electrospinning of depolymerized chitin solutions is a widely employed bottom-up technique.[38,39] In electrospinning, a high voltage applied to a solution-filled capillary creates an electric field that overcomes surface tension, forming a polymer jet. Upon contact with a substrate, the jet solidifies into nanofibers. Chitin is often depolymerized with gamma radiation and dissolved in solvents like 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) to improve solubility. Alternatively, ionic liquids[40–42] like 1-butyl-3-methylimidazolium chloride[40] or 1-allyl-3-methyl-imidazolium bromide[41,43] can be used to dissolve chitin at elevated temperatures. The dry-jet-wet-spinning method has also been employed to spin chitin dissolved in acetate salt solutions into microscale fibers.[43] Huang *et al.*[44] demonstrated the direct spinning of pure chitin microfibers from NaOH–urea solutions after freeze–thaw cycles (Fig. 7).

Self-assembly methods for producing ChNFs from chitin solutions in organic solvents without electrospinning have been developed[45–47] (Fig. 8). These methods involve dissolving squid pen β-chitin in either HFIP or LiCl/*N*,*N*-dimethylacetamide (DMAC) to disrupt hydrogen bonds. Self-assembly is then initiated by solvent evaporation (HFIP) or precipitation (LiCl/DMAC). While LiCl/DMAC yields nanofibers of various sizes (3, 6, and 10 nm), HFIP-chitin solutions form monodispersed ChNFs with diameters of 3 nm (Fig. 8a–c). Interestingly, these self-assembled nanofibers exhibit the α-chitin crystal structure, which is more energetically favorable. Notably, these 3 nm α-ChNFs closely resemble those found in crustacean shells and arthropod cuticles, serving as versatile building blocks for biomimetic ChNF assemblies. The flowchart in Fig. 9[36] illustrates various bottom-up synthesis procedures for ChNFs. Table 1 provides a comparative analysis of these different bottom-up synthesis methods, outlining their respective challenges, applications, and cost-effectiveness.

3 Preparation of ChNFs from different sources

Several methods are employed to prepare ChNFs. The ionic gelation method involves dissolving chitosan in an acidic solution and adding a cross-linking agent like sodium tripolyphosphate (TPP) to induce nanoparticle formation through ionic interactions. In the emulsion-droplet coalescence method, a chitosan solution is mixed with an oil phase and subjected to ultrasonication to produce chitosan nanoparticles. The reverse microemulsion method involves stimulating a water-in-oil microemulsion containing chitosan and a cross-linking agent to form nanoparticles. Lastly, the coacervation method entails mixing chitosan with another polymer in water and inducing nanoparticle formation through a pH change or salt addition.

Table 1 Different bottom-up synthesis methods of ChNFs and their challenges, application, and cost-effectiveness

Method	Advantages	Challenge	Principal application	Environmental impact	Cost	Ref.
Electrospinning	High surface area, scalable, fine control	Specific solvent requirements, costly	Textiles, filtration, biomedical	Moderate (solvent use, energy)	Moderate to high (equipment costs)	48–50
Self-assembly	Low-cost, green, simple process	Limited control over fiber morphology	Sustainable applications, bio-based products	Very low (green process)	Low (simple process, less equipment)	51 and 52
Hydrothermal/solvothermal	High crystallinity, controlled morphology	High-energy requirements, expensive	Large-scale production, high-performance materials	Moderate (energy-intensive)	High (specialized equipment, energy)	53 and 54
Biomimetic synthesis	Precision, functionalization, biocompatibility	High cost, scalability issues, complexity	Biomedical, sustainable packaging, cosmetics	Low	Hight	55 and 56
Enzymatic hydrolysis	Biodegradable, environmentally friendly	High cost of enzymes, slow process	Biodegradable applications, small-scale	Very low (biodegradable enzymes)	High (enzyme cost, slow process)	57 and 58

3.1 ChNFs from crab shell

Crab shells, composed primarily of 25–30% chitin, 25% protein, and 40–50% calcium carbonate,[59] exhibit a hierarchical structure with multiple layers (Fig. 10). ChNFs are encased in protein layers, forming a crystalline structure. Chitin synthesis can be achieved through traditional chemical or enzymatic approaches.[60] ChNFs have been successfully extracted from crab shells using a disintegration method.[5,61] Following purification through various conventional chemical treatments, the shells underwent mechanical processing to yield ChNFs. These NFs exhibited remarkable homogeneity, with a width of approximately 10 nm (Fig. 11).

3.1.1 Extraction and deacetylation of chitin NFs from the speckled swimming crab shells. The extraction of ChNFs from speckled swimming crab shells involves a multi-step process encompassing deproteinization, demineralization, depigmentation, and mechanical disintegration, as shown in Fig. 12.

Initially, the crab shells are physically pulverized into fine powders using a high-speed rotor mill. These powders are then subjected to sequential treatments with NaOH, HCl, and ethanol solutions to eliminate proteins, minerals, and pigments. This process yields chitin with a 12 wt% yield. Subsequently, ChNFs are produced from the purified chitin through mechanical disintegration methods, such as wet grinding and high-pressure homogenization. The purified wet chitin is diluted to a 1 wt% suspension in deionized water under vigorous mechanical stirring. This suspension is then processed using a grinder equipped with grinding stones at room temperature, followed by passage through a high-pressure homogenizer with Z-shaped interaction chambers. High-pressure homogenization is repeated at controlled temperatures, ensuring the preservation of the ChNFs' original chemical and crystalline structures. Partial deacetylation of the ChNFs is achieved through treatment with a NaOH solution under vigorous mechanical stirring at elevated temperatures. The resulting ChNF and deacetylated ChNF (dChNF) suspensions are dialyzed against deionized water until a neutral pH is attained. Finally, the suspensions are diluted and stored for future use. This straightforward yet effective process enables the extraction of substantial quantities of homogeneous ChNFs from crab shells.[5,24]

3.1.2 Chitin synthesis from crab shell by enzymatic hydrolysis of crab gill proteins. An alternative method for chitin extraction, developed by PINRO for integrated processing of king crab shells, involves the recovery of chitin and enzymatic protein hydrolysates from crab gills and shells.[60] This contrasts with the traditional chemical method described earlier, which focuses on extracting and deacetylating ChNFs from speckled swimming crab shells.

The enzymatic method begins with physically grinding crab gills and mixing them with water (1 : 2 ratio). The mixture is heated to 50 °C under stirring, followed by the addition of an enzyme preparation in a 1 : 0.06 ratio. Enzymatic hydrolysis proceeds for 6 h at 50 °C, maintaining a pH of 6.5–7.0. The degree of protein hydrolysis (DH) is monitored, and upon reaching the maximum DH, enzyme activity is inhibited by

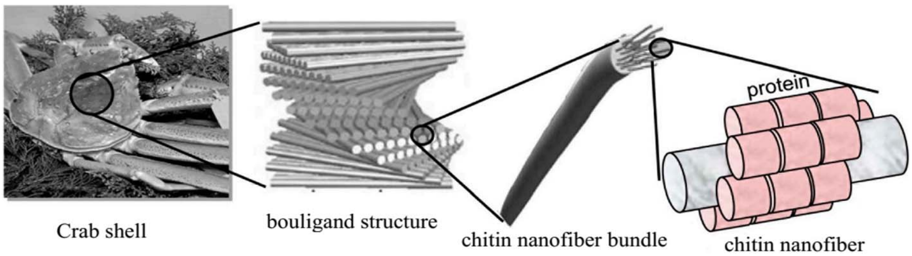

Fig. 10 Schematic presentation of the exoskeleton structure of crab shells.[1]

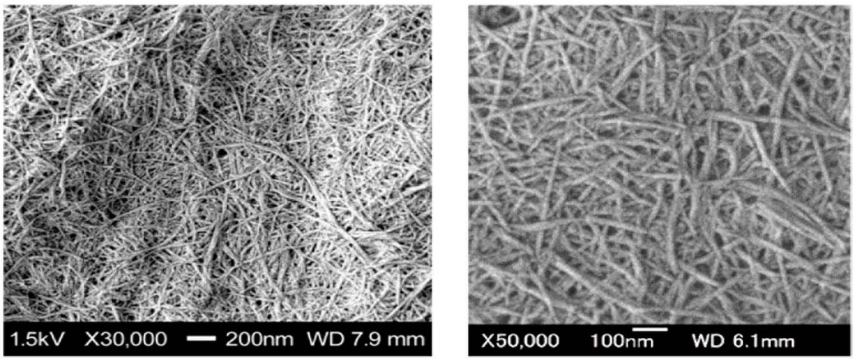

Fig. 11 SEM images of ChNFs from crab shell after grinder treatment.[1]

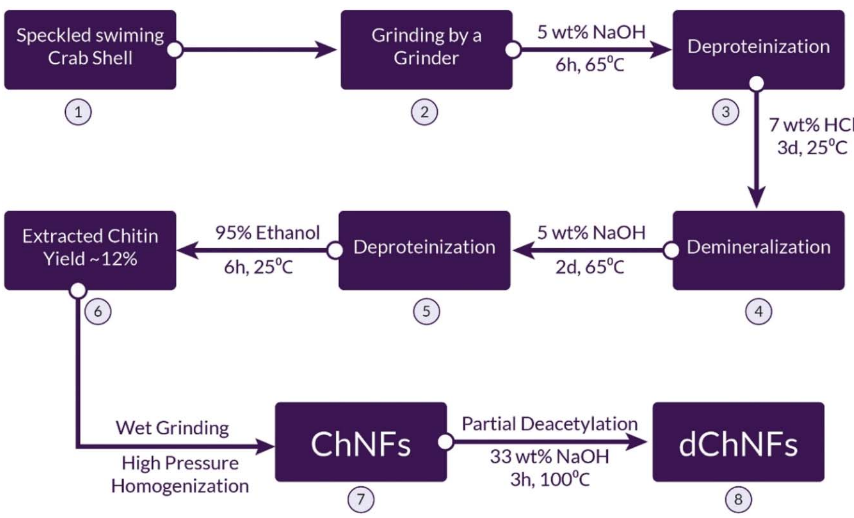

Fig. 12 Schematic illustration of the preparation of ChNFs and dChNFs from speckled swimming crab shells.[24]

heating the mixture to 95 °C, followed by cooling to 30 °C. Centrifugation is employed to separate the precipitate, which is then washed multiple times. The resulting precipitate serves as the starting material for chitin preparation, following the steps outlined in the chemical approach, with the exception of the initial deproteinization using a 4% NaOH solution.

Wet chitin samples obtained through both methods are dried at a maximum temperature of 60 °C. The transparent protein hydrolysate solution resulting from the enzymatic method is further dried using a lyophilizer.[60]

3.1.3 Chitin synthesis from crab shells by fermentation with microorganisms. Hajji *et al.*[62] compiled research on the fermentation-based synthesis of chitin from king crab shells, focusing on the use of six *Bacillus* strains that produce proteases: *B. subtilis* A26,[63] *B. mojavencis* A21,[64] *B. pumilus* A1,[65] *B. amyloliquefaciens* An6,[66] *B. licheniformis* NH1,[67] and *B. cereus* BG1.[67,68] Fresh crab shells, obtained as byproducts from a fishery processing plant in Sfax, Tunisia, were washed, dried, processed (sieved to 2–5 mm), and stored until use.

Crab waste fermentation was performed under previously established conditions using the *Bacillus* strains. Inocula were routinely cultured in a Luria-Bertani (LB) broth medium.[62,69] Fermentation occurred in 500 mL jars containing 100 mL of medium of 3% (w/v) crab shell waste, with or without 5% (w/v) glucose. The initial pH of the broth was adjusted to 7.0. Following sterilization, inoculums were added, and cultures were incubated for 5 days at 37 °C with shaking (200 rpm). Cultures were centrifuged, and the resulting fermented crab supernatants (FCSs) were freeze-dried and stored at −20 °C for further analysis. The FCSs were evaluated for composition, antioxidant activity, and antibacterial properties. Fermented crab waste was separated, washed, and dried for chitin recovery.[62]

3.2 ChNFs from prawn & shrimp shell

Crustacean shells primarily consist of chitin, calcium carbonate, proteins, lipids, and pigments. Chitin extraction involves deproteinization, demineralization, and removal of lipids and pigments. These steps, along with the subsequent conversion to chitosan, can be achieved through chemical or biological methods, such as microbial fermentation and enzymatic reactions. Chitosan, a partially acetylated form of chitin with a degree of deacetylation (DDA) around 50%, becomes soluble in acidic aqueous solutions.[1,70]

The hierarchical structure of prawn shells allows for the application of ChNF extraction methods similar to those used for crab shells. The ChNFs with a uniform structure and high viscosity can be obtained from various prawn species, including *Penaeus monodon*, *Marsupenaeus japonicas*, and *Pandalus eous Makarov*. These nanofibers, derived from prawn shells, exhibit a thickness of 10–20 nm, similar to crab shell nanofibers (Fig. 13). The predominance of the finer exocuticle in prawn shells, compared to the coarser endocuticle in crab shells, facilitates easier fibrillation of chitin from prawn shells.[71]

Chitin can also be synthesized from shrimp shells (Fig. 14). This process involves demineralization using a diluted HCl solution, followed by deproteinization with NaOH. The resulting crude chitin is dehydrated with ethanol and dried. Crude chitosan is then prepared by treating the chitin with NaOH at elevated temperatures.[72] The purification of chitosan from prawn exoskeletons for potential medicinal use involves the removal of insolubles, reprecipitation with NaOH, and demetallization. This approach has successfully yielded chitosan

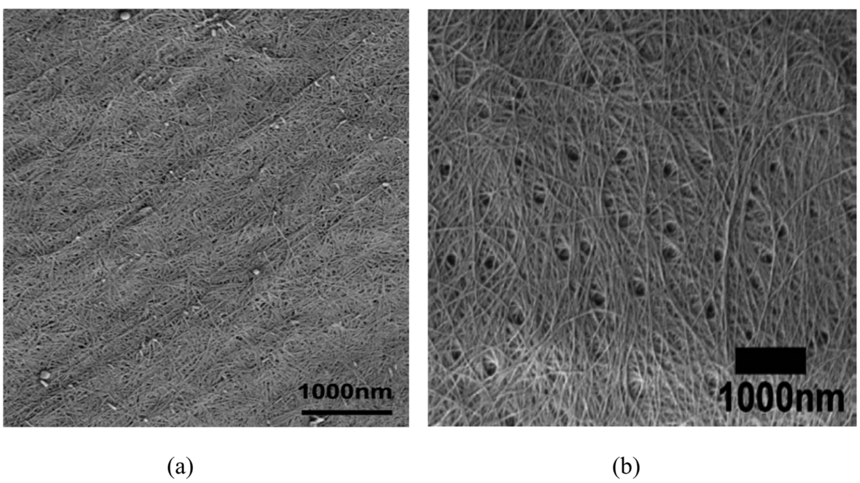

Fig. 13 (a) FE-SEM micrograph of ChNFs from black tiger prawn shells; (b) FE-SEM micrograph of the surface of the black tiger prawn shell after removing the matrices.[28]

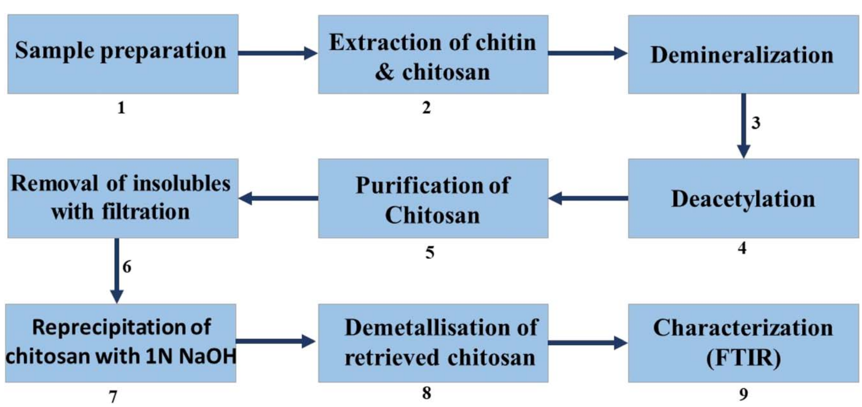

Fig. 14 Synthesis of chitin from shrimp and prawn.[74]

with a 35.49% yield, demonstrating its potential as a pharmaceutical excipient.[73] Chitin nanofibers can be derived from these chitins using various top-down and bottom-up processes, which are explored in this article.

Another method for producing ChNFs from shrimp involves dissolving chitin and subsequently electrospinning it into NF materials. Electrospinning, a simple, cost-effective, and high-throughput technique, creates nanofibrous scaffolds that mimic the natural extracellular matrix.[75,76] It is the most widely used method for NF production due to its ease of operation. The electrospinning system typically consists of a grounded collector, a spinneret, and a high-voltage power source. A precursor solution is fed into the spinneret, forming a pendant drop that transforms into a Taylor Cone under high voltage. The resulting liquid jet stretches and whips, leading to solvent evaporation and fiber collection on the target. Electrospinning equipment is rapidly being commercialized,[74,77] and various modifications to the traditional method have been developed to overcome its limitations.[74,78]

3.3 ChNFs from mushroom

Mushroom cell walls also contain ChNFs, forming complexes with glucans.[78–80] A variety of mushroom species, namely *Pleurotus eryngii* (king trumpet mushroom), *Agaricus bisporus* (common mushroom), *Lentinula edodes* (shiitake), *Grifola frondosa* (maitake), *Hypsizygus marmoreus* (bunashimeji), *Armillariella mellea* (honey mushroom), and *Morchella esculenta* (yellow morel), underwent a systematic series of chemical treatments. These treatments were specifically designed to eliminate inherent proteins, pigments, glucans, and minerals from the fungal biomass.[6,81] Following the purification process, the resultant material was subjected to nano-fibrillation utilizing an acetic acid-impregnated grinder, yielding uniform and thin ChNFs (Fig. 15).

Mushroom-derived ChNFs, unlike to those from crab and prawn shells, are characterized by the formation of complexes with glucans on their surface. Due to the carbohydrate nature of both glucan and chitin, the complete removal of glucans from chitin is challenging through chemical treatment alone. The

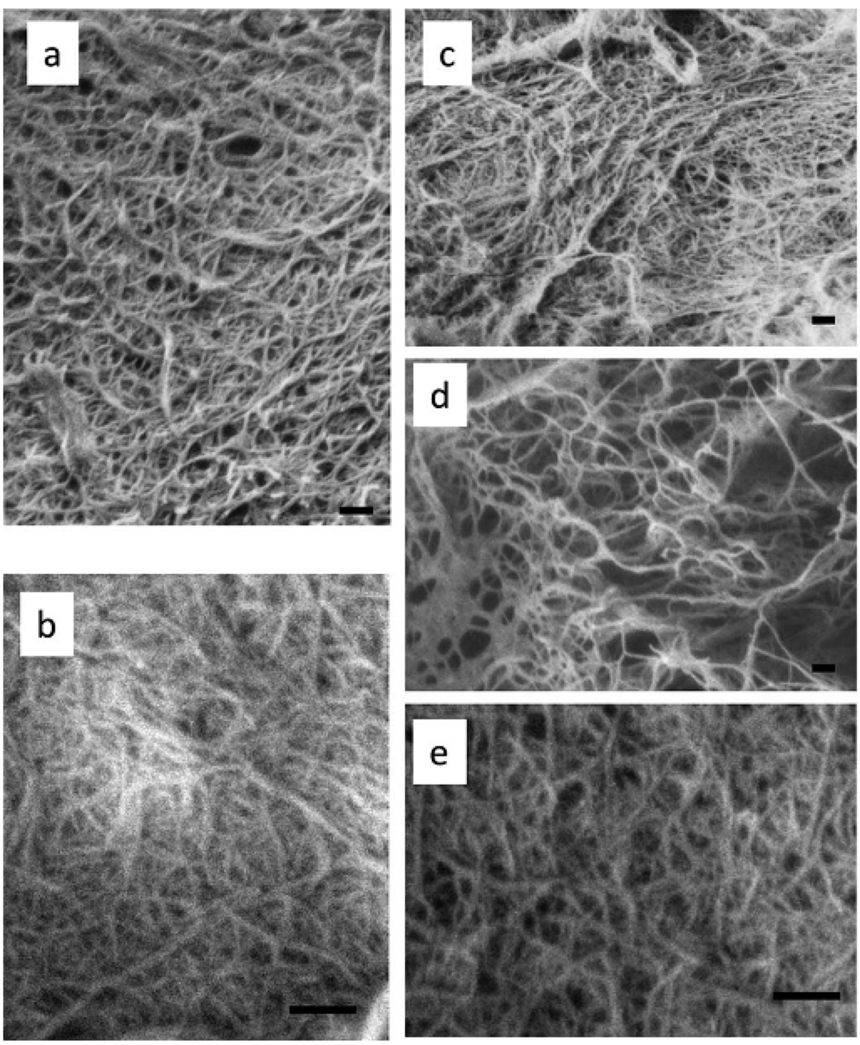

Fig. 15 FE-SEM micrographs of ChNFs from (a) *Pleurotus eryngii*, (b) *Agaricus bisporus*, (c) *Lentinula edodes*, (d) *Grifola frondosa*, and (e) *Hypsizygus marmoreus*. The scale bars are 200 nm in length.[6]

glucan content and resulting nanofiber width varied among mushroom species, ranging from 20 to 28 nm. Despite exhibiting typical α-chitin crystal patterns in X-ray diffraction, the relative crystallinity indices of these nanofibers decreased with increasing amorphous glucan content. These findings expand the repertoire of dietary nanofibers and highlight the potential of mushroom-derived nanofibers for various applications, from novel food ingredients to medical applications.[82–84]

Zhang *et al.*[85] adapted the abovementioned process with minor modifications to extract ChNFs from shiitake stripes, achieving a purity of over 98% and a diameter of 9 nm. Alternative methods have been reported to extract ChNFs from mushrooms without acid treatment. For instance, mechanical agitation and hot water treatment, followed by alkali treatment with NaOH, were employed to extract ChNFs from Agaricus bisporus, yielding 25.4% and 15% from the stalk and cap, respectively.[86,87] This method has been extended to other mushroom species, including *Pleurotus ostreatus*, *Lentinula edodes*, and *Flammulina velutipes*.[87] Aitor Larrañaga *et al.*[88] utilized a top-down approach involving mechanical fibrillation, removal of water-soluble components, and deproteinization to isolate ChNFs from *Agaricus bisporus* with a crystallinity of 59.1%.

Another method (Fig. 16) involves extracting ChNFs from *Agaricus bisporus* cultivated in a liquid culture medium supplemented with various salts, hormones, and trace metals.[89] After removing unwanted materials, the resulting chitin is ground to produce ChNFs.

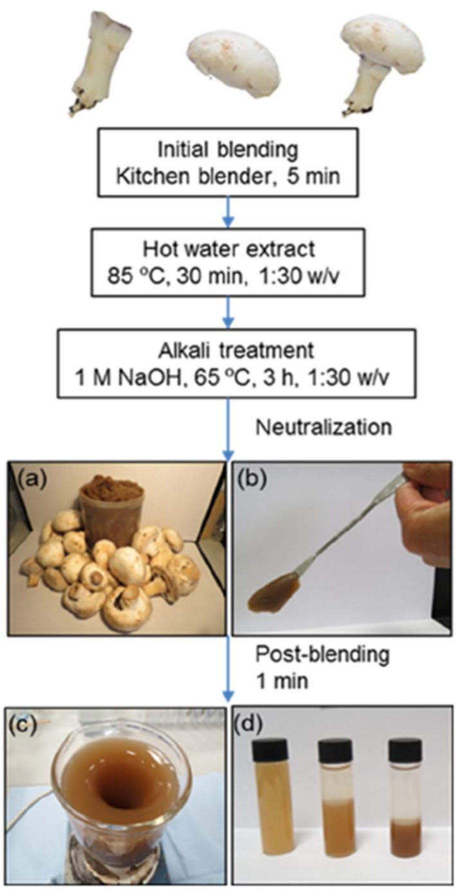

Fig. 16 Schematic representation of the extraction of ChNFs from the commonly cultivated *Agaricus bisporus*: (a) extraction from 3 kg of whole mushrooms resulted in approximately 42 g of nanofibers. (b) A 3% w/v extract was obtained through chemical extraction. (c) A 0.8% w/v whole mushroom suspension, dispersed within one minute of blending and never dried, was used for nanopaper production. (d) Stability assessment of the 0.8% w/v whole mushroom chitin suspension after 7 days: left – the never-dried suspension; middle – resuspension of a freeze-dried sample after rapid freezing with liquid nitrogen; right – resuspension of a freeze-dried sample after slow freezing in a conventional freezer.[86]

3.4 ChNFs from squid pen

Two distinct crystalline forms of chitin exist in nature: α- and β-chitin, differentiated by antiparallel and parallel chain-packing modes, respectively.[90,91] While α-chitin is widely distributed in nature, β-chitin is uniquely found in squid pens. Fan and Isogai *et al.*[91] pioneered the development of ChNFs from squid pen β-chitin, obtaining NFs with a 3–4 nm length and a high aspect ratio without the need for chemical modification. The lower crystallinity, parallel chain packing, and relatively weak intermolecular forces of β-chitin contribute to the facile preparation of ChNFs.

Various methods have been employed to synthesize β-ChNFs from squid pens. A common approach involves cleaning, cutting, and grinding the squid pen, followed by deproteinization and pH neutralization to extract pure chitin and lower the high pH resulting from the alkalization process. Another procedure involves creating a slurry by mixing and oxidizing the squid pen with ammonium persulfate and heating it, followed by suspension in water, pH adjustment, and ultrasonication to extract ChNFs.[92,93]

A distinct method utilizes high-temperature water treatment to extract β-ChNFs. In this approach, the squid pen content, water, and molten KNO_3–$NaNO_3$ salts are combined in a reactor at high temperatures (150 °C, 200 °C, 250 °C, and 300 °C) and then cooled. Subsequently, the residue is converted into a ChNF dispersion and disintegrated by a 'Starburst' system, which is a high-pressure water jet system.[94,95]

Alternatively, the 'Starburst' system can be used to prepare a slurry from the solid residue obtained through high-temperature water treatment and disintegrate it into nanofibers. This system has proven effective in converting acid-to-base and base-to-acid treated β-chitins into ChNFs, demonstrating its versatility in handling different pretreatment sequences.[96]

3.5 ChNFs from commercial chitin powder

A challenge in ChNF production is maintaining extracted chitin in a wet state to prevent inter-fibrillar coagulation, which hinders commercialization efforts. To address this, researchers have developed a simplified method for producing ChNFs from commercially available dry chitin powder. This method involves dissolving the dry chitin in acidic water and subjecting it to grinding. The commercial chitin, composed of NF aggregates, readily disintegrates into homogeneous NFs due to electrostatic repulsion between the cationized amino

Table 2 Comparison of chitosan nanofibers (ChNFs), nanocellulose (NCs), and silk fibroin

Type	Properties	ChNFs	Nanocellulose	Silk fibroin	Ref.
Structural properties	Size	2–5 nm	10–90 nm	150–400 nm	5, 100 and 101
	Surface area to volume ratio	High surface area due to the nanoscale size and fibrillar structure	Slightly higher due to narrower fibers	Lower than cellulose or chitin due to larger aggregates	102 and 103
	Crystal structure	α-Chitin crystal structure with antiparallel arrangement	Cellulose Iβ structure in native cellulose	β-sheet crystalline regions in its native form	104–106
Mechanical properties	Tensile strength	Partially deacetylated ChNF films exhibit the highest tensile strength of ∼140 MPa	Cellulose nanofiber green composites can achieve tensile strengths up to 90 MPa, comparable to glass-fiber-reinforced plastics	Ultrathin silk fibroin films have high tensile strength and toughness due to their self-reinforcing microstructure	107, 108 and 109
	Young's modulus	Chitin nanopapers from mushroom extract have a Young's modulus of around 7 GPa	The Young's modulus of cellulose nanofibers from different sources ranges from 102 to 131 GPa, as measured by atomic force microscopy	Uniaxial extension of regenerated silk fibroin films increases their Young's modulus from 2.7 to 3.5 GPa	110 and 111
Electrical properties	Electrical conductivity or resistivity	Insulating but can be modified for conductivity using composites	Insulator; conductive properties enhanced when hybridized with graphene	Limited conductivity but can function as a dielectric layer	112, 113 and 114
Thermal properties	Thermal conductivity	Chitin nanofiber films exhibited in-plane thermal conductivity of 0.73–0.82 W m^{-1} K^{-1}, with surface amino groups influencing conductivity	Nanocellulose filaments fabricated by flow-focusing can exhibit thermal conductivity up to 14.5 W m^{-1} K^{-1}, much higher than cellulose nanopaper or nanocrystals	Single silk fibroin fibers exhibit an axial thermal conductivity of approximately 0.775 W m^{-1} K^{-1} at room temperature, which is significantly higher than most textile fibers	115–117
	Heat capacity	Not favourable	Moderate	Moderate	118–120
	Thermal stability	ChNFs start decomposing at 33 °C	Chemical pretreatments can enhance thermal properties, with NaOH/urea/thiourea-treated nanofibers demonstrating thermal degradation onset at 270 °C and maximum degradation at 370 °C	Silk fibroin decomposes at around 348 °C	121 and 122
	Thermal expansion coefficient	Low	Low	Slightly higher	123
Biological properties	Biocompatibility	ChNFs demonstrate excellent biocompatibility, promoting cell proliferation and collagen deposition, which are crucial for wound healing	Critical biocompatibility	Superior biocompatibility	124 and 125
	Antibacterial or antiviral activity	Chitin-based materials demonstrate over 99.95% bacteriostasis against pathogens like *Staphylococcus aureus* and *Escherichia coli*, making them effective in medical and civil applications	Its large surface area and porous structure facilitate effective interactions with bacteria, disrupting their membranes and inhibiting proliferation	Silk fibroin membranes combined with polyhexamethylene biguanide (PHMB) or silver oxide nanoparticles effectively inhibit *Staphylococcus aureus* and *Escherichia coli*	126–128

Table 2 (*Contd.*)

Type	Properties	ChNFs	Nanocellulose	Silk fibroin	Ref.
	Biodegradability	Chitin is highly biodegradable, breaking down into simple organic acids, which supports bacterial growth due to its favorable carbon : nitrogen ratio	Nanocellulose, derived from cellulose, is also biodegradable and exhibits excellent mechanical properties, making it suitable for various applications	Silk fibroin is known for its biocompatibility and biodegradability, although its degradation rate can be slower compared to chitin	129 and 130
Surface properties	Adsorption and desorption behavior	High for all-purpose	Medium to high	High for heavy metals	131 and 132
Environmental properties	Photocatalytic degradation of pollutants	Chitin-based composites, particularly when integrated with TiO_2, exhibit improved photocatalytic activity due to reduced band gap energy and enhanced reactive sites	The three-dimensional structure of these aerogels provides a large surface area, promoting effective photocatalytic reactions through increased active sites	Combined with metal oxide nanoparticles like ZnO and TiO_2, the degradation efficiency of various organic pollutants, including pesticides and dyes, is enhanced under solar irradiation	133–136
Water purification potential	Excellent adsorption properties for heavy metals in water treatment	Strong capability for dye and heavy metal removal	Moderate potential for water purification, enhanced with chemical modifications	Water purification potential is medium	137 and 138
Environmental stability	Stable in mildly acidic, basic, and oxidative conditions but degrade under extreme environments, such as strong oxidants or high temperatures	Highly stable in aqueous and neutral environments. Stability decreases under strongly acidic or basic conditions but can be enhanced by cross-linking	Stable in physiological environments but prone to enzymatic degradation in biological systems. Combining it with nanocellulose enhances its environmental stability	Environmental stability	139 and 140

groups on the crystalline surfaces. Various organic acids can further enhance this disintegration process. The advantage of commercial chitin is the ability to obtain large quantities of ChNFs rapidly.

In addition to grinding, the Star Burst instrument, a high-pressure waterjet system, has proven effective in nano-fibrillating dry chitin.[97,98] This system, equipped with a ball-collision chamber, subjects chitin in aqueous acetic acid to high-pressure ejection through a small nozzle. Multiple treatments progressively reduce the thickness of the resulting NFs.

4 Comparison of ChNFs with other similar nanofibers

The ChNFs, nanocellulose (NFC), and silk fibroin (SF) are three naturally occurring biopolymers attracting significant interest due to their potential for creating sustainable and high-performance materials. Each possesses unique structural characteristics and functionalities, making them ideal candidates for various applications, particularly in the development of biocomposites. This section will explore their individual properties and the synergies achieved by combining them. Chitin, the second most abundant natural polymer after cellulose, boasts a semi-crystalline structure stabilized by hydrogen bonding and van der Waals interactions. Processing it into nanofibers *via* techniques like acid hydrolysis and high-pressure homogenization enhances its mechanical properties (strength and stiffness) and expands its applicability in composites. Derived from cellulose, a major component of plant cell walls, nanocellulose exhibits a highly crystalline structure, translating to exceptional mechanical strength and flexibility. It can be processed into two primary forms: nanofibrils (elongated, high-aspect-ratio) and nanocrystals (rod-like, high crystallinity). These forms are crucial in enhancing composite materials' mechanical and optical properties. Silk fibroin, a protein-based biopolymer extracted from silk cocoons, possesses a unique hierarchical structure for its excellent mechanical properties (high tensile strength) and biocompatibility. Its ability to transition from a random coil conformation to a β-sheet structure is crucial for its efficient integration into composite materials. Combining these biopolymers offers significant advantages when compared to their individual use.

When incorporated into an SF matrix, ChNFs significantly improve the composite's mechanical performance by reinforcing the structure and enhancing strength and flexibility. The addition of cellulose nanocrystals to composites can significantly improve tensile strength and elongation at break. Silk fibroin contributes to biocomposites overall ductility and toughness, making it an ideal matrix material for embedding other nanofibers.

Due to their unique properties, these biopolymers hold promise for various applications. Their biocompatibility and mechanical properties make them suitable for biomedical applications like wound dressings and drug delivery systems. Additionally, their renewability and biodegradability make them attractive for use in textiles, medicine, and even environmental decontamination.[99] Chitin and chitosan nanofibers are highly usable, except for thermal and some chemical limitations, they carry excellent properties. A concise comparison of ChNFs, NCs, and silk fibroin is presented in Table 2.

5 Versatile applications of ChNFs

The ChNFs, derived from the second most abundant natural polymer, chitin, have emerged as a promising biomaterial due to their attractive properties such as biodegradability, biocompatibility, and high mechanical strength.[112] The applications of ChNFs span various fields, including packaging, wastewater treatment, food, agriculture, cosmetics, and biomedicine.[141] In the biomedical domain, ChNFs are particularly suitable for tissue engineering, drug delivery, wound dressing, and cancer diagnostics due to their non-toxicity, biocompatibility, and biodegradability. Other applications include cosmetics, food, agriculture, paper finishing, and solid-state batteries. Despite its versatility, chitin's poor solubility presents a challenge, often addressed through modification or functionalization. While traditionally focused on non-biomedical fields, recent research has increasingly highlighted the potential of chitin and ChNFs in biomedical applications.[37]

5.1 Biomedical applications

ChNFs, due to their unique properties of biocompatibility, biodegradability, and non-toxicity, have become highly desirable for biomedical applications. In the past fifty years, a wide range of chitin-based materials, including gels, membranes, scaffolds, nanofibers, microfibers, and nanoparticles, have been developed for use in tissue engineering, wound dressing, drug delivery, and cancer recognition.[37,141] A study depicted the combination of FfAA11 with a chemical method designed to transform resistant chitins into functionalized materials. The approach employs oxyma-assisted click chemistry using ethyl (hydroxyimino) cyanoacetate, enabling rapid surface modifications. These modifications facilitate the incorporation of a fluorescent probe, a peptide, and gold nanoparticles, thereby enhancing the functionalization of chitins for various applications in materials science and biomedicine. This methodology demonstrates the versatility of click chemistry in surface engineering and its potential for developing advanced materials with tailored properties. The process is environmentally friendly, producing no toxic by-products or waste organic solvents, representing a greener approach to producing chitin-based biomaterials.[142]

5.2 Tissue engineering

ChNFs have garnered significant attention in tissue engineering due to their structural and functional resemblance to the natural extracellular matrix (ECM).[37] These nanofibers have been extensively explored as scaffoldings to support the regeneration of human tissues. Tissue engineering aims to restore, replace, maintain, or enhance the function of damaged tissues or organs by utilizing living cells to create biological

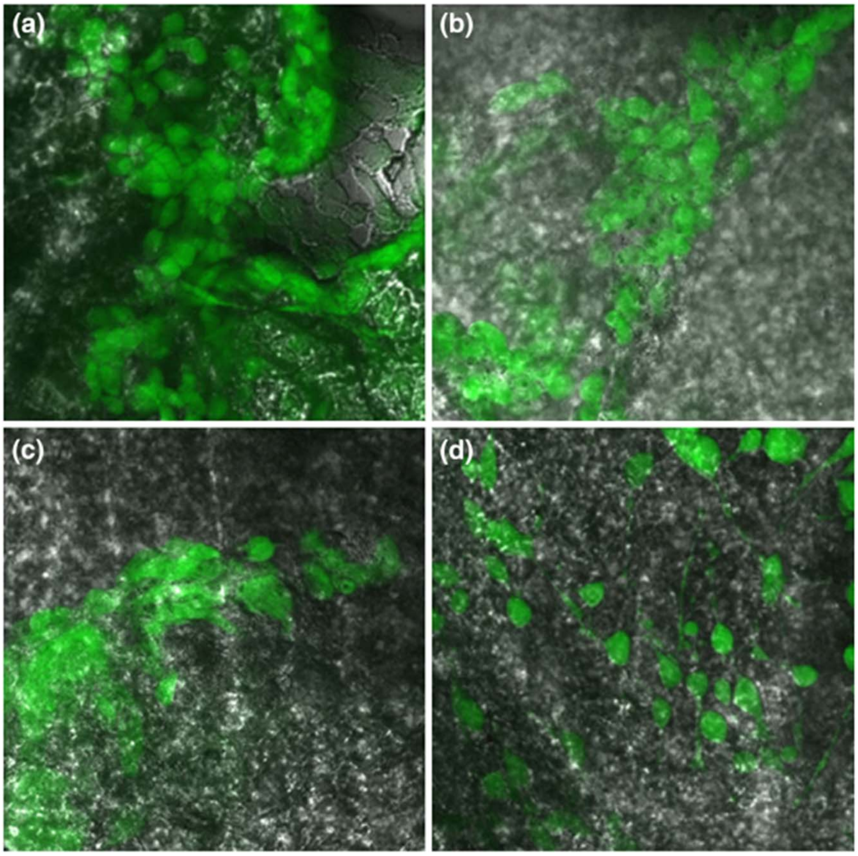

Fig. 17 (a) The growth of NIH/3T3 fibroblast cells on the chitin membrane prepared from RG and (b–d) the growth of NIH/3T3 fibroblast cells on different samples of chitin membranes prepared from SG.[149]

substitutes.[143,144] While chitin possesses low mechanical properties, its potential for bone tissue engineering can be improved by incorporating biomaterials such as hydroxyapatite (HA) or bioactive glass ceramics.[141] Carboxymethyl chitin (CMC)/PVA blend nanofibrous scaffolds and α-chitin/nano bioactive glass ceramics (nBGC) composite scaffolds have also demonstrated promise for tissue engineering applications due to their bioactivity and non-toxicity.[145] Recent research has highlighted the potential of β-chitin hydrogel/nano-hydroxyapatite (n-HAp) nanocomposite scaffolds, synthesized *via* freeze-drying, for bone tissue engineering. These scaffolds exhibit high porosity (70–80%), controlled biodegradation (30–40%), and improved protein adsorption.[146] The incorporation of nano ZrO_2 into chitin-chitosan scaffolds has also been shown to enhance osteogenesis, further expanding the possibilities for bone tissue engineering.[147]

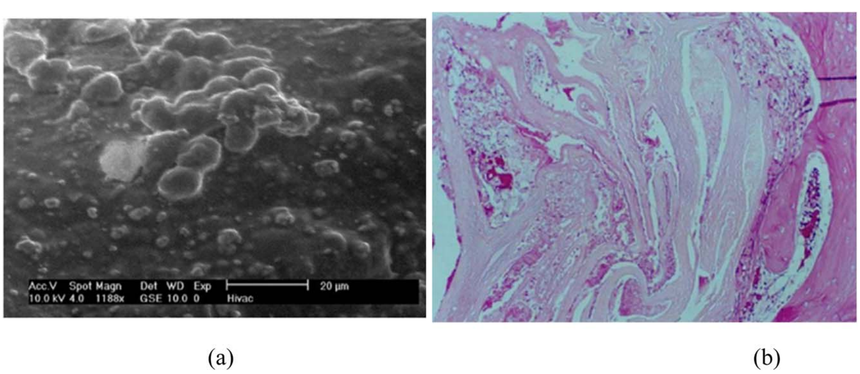

Fig. 18 (a) SEM image of osteoblasts proliferated on the surface of a 25% HA–chitin thin film after 2 weeks. (b) Micrograph image of cell-free porous HA–chitin matrix after 2 months implantation (Rabbit femur model).[151]

Chitin's biocompatibility and bioactivity can be augmented by the addition of silica. Chitin/nano-silica composite scaffolds have displayed bioactivity in simulated body fluid (SBF), biocompatibility with MG63 cell lines, and enhanced swelling ability, making them suitable for bone tissue engineering.[148] Furthermore, α and β-chitin/gelatin membranes have been developed for tissue engineering applications. Chitin regenerated hydrogel (RG) and swelling hydrogel (SG) were prepared using α-chitin and β-chitin, respectively. These hydrogels were then mixed with gelatin and *N*-acetyl-D-(+)-glucosamine to create chitin/gelatin membranes. Thermal stability studies revealed that RG-based membranes exhibited superior thermal stability compared to SG-based membranes. Both types of membranes supported the growth of NIH/3T3 fibroblast cells, highlighting their potential in tissue engineering (Fig. 17).[149,150]

Chitin–chitosan/nano TiO_2 composite scaffolds, synthesized through lyophilization, have also shown promise. Increasing TiO_2 content led to reduced pore size, non-toxicity to various cell lines (MG-63, L929, and hMSCs), enhanced thermal stability, bioactivity, swelling, and degradation. These nanocomposite scaffolds can improve cell seeding and tissue growth, making them valuable for bone regeneration.[147] Hydroxyapatite (HA)–chitin materials, fabricated by incorporating HA into chitin solutions, have demonstrated non-cytotoxicity, porosity, and enhanced degradation. These properties make them suitable for bone substitution due to their ability to promote the ingrowth of surrounding tissues (Fig. 18a and b).[146]

In tissue engineering, engineered tissues require enhanced cellular and ECM organization for optimal function.[152,153] Cell reorganization is influenced by factors such as topography, mechanical properties (stiffness, elasticity, viscosity), and interactions with the ECM.[154] Concentration gradients of immobilized growth factors and ECM molecule alignment also play crucial roles in cellular organization.[153] Substrates with controlled micro and nanopatterns have been developed to promote cellular organization, elongation, and orientation in engineered tissue.[155] These substrates enable the production of functional and highly ordered cell sheets that can be easily detached and delivered to host tissues *via* enzymatic degradation or thermal stimulus.[156] However, engineered cell sheets are often mechanically weak and challenging to handle, necessitating the use of a support platform for delivery to host tissues.[157] These substrates need to be thin, flexible, robust, and easy to handle to ensure conformal contact with the target tissue.[158] Structural biopolymers such as chitin, chitosan, and collagen are gaining popularity as support platforms due to their biocompatibility, nontoxicity, cytocompatibility, tunable biodegradability, and mechanical strength.[159]

Transparent, robust, and ultra-thin ChNF substrates with tunable and superior mechanical properties demonstrate significant promise as potential substrates in tissue engineering.[160] Hassanzadeh *et al.*[160] prepared and investigated both supported and free-standing micropatterned substrates composed of self-assembled ChNFs. These substrates, being mechanically robust, flexible, biodegradable, and easy to manipulate, show potential in creating complex tissue structures for regenerative medicine and tissue engineering applications, including myocardial repair, where the chitin substrate could provide mechanical support to damaged tissue during regeneration.[152] The fabrication process involved pouring a chitin/HFIP solution onto a mold covered with a glass slide and drying it overnight for supported substrates, while free-standing substrates were obtained from more concentrated solutions.[160] The resulting cell sheets were mechanically robust, flexible, and easily manipulated.[160] Fig. 19 illustrates approaches to producing transparent, ultra-thin (<10 μm), mechanically robust, and flexible self-assembled ChNF microplate substrates for tissue engineering.[160]

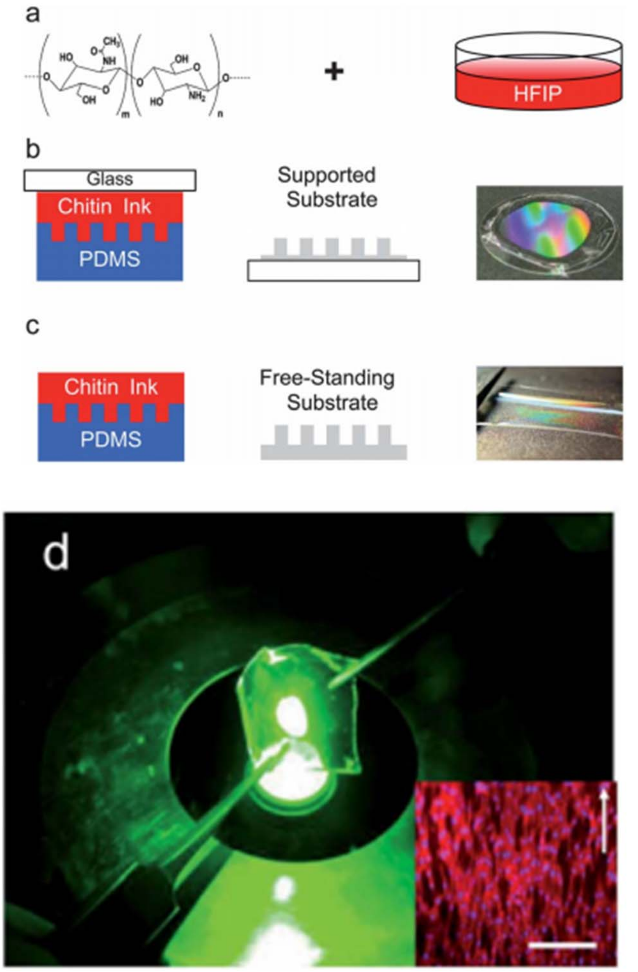

Fig. 19 ChNF-based micropatterned substrate fabrication process. (a) Chitin/HFIP solution (0.1% w/w) was poured on top of the (b) mold covered with a glass slide to create a supported substrate after drying overnight and substrate optical image with the diffraction pattern. (c) Thicker films were obtained from more concentrated solutions (0.2% w/w) to create free-standing substrates, which were robust and easy to handle. The optical image demonstrated the diffraction pattern on the free-standing chitin film. (d) ChNF substrates are transparent and afford optical inspection (Inset). Fluorescence images of the actin cytoskeleton of the cells on G2 show the entire coverage and alignment of cells within the direction of the patterned features. The white arrow on the right corner of the inset image indicates the direction of the patterns. Scale bar 100 mm.[160]

Table 3 ChNFs in biologically active matrices and TE applications

Matrix	Chitin origin & isoform	Additives	Biomaterials	Role of CHNFs	TE application	Ref.
Chitin			Electrospun nano-fiber mats	No-inflammation *in vivo* improve cell attachment and spreading	Oral mucosa	38
	Alpha isoforms		Nanofiber mat	Surface deacetylation increases cell adhesion and proliferation	Biomedical materials	165
	Crab alpha isoform		Wet-spinning microfibers	High water sorption capacity supports the proliferation of rat cardiac myoblasts and mouse bone osteoblasts	Cardiac bone	21
	Alpha isoform	Carbon nanotubes	Hydrogels	Enhance mechanical properties hemocompatible biocompatible to neuronal, and Schwann cells improve neuronal cell behavior	Nerve	166
	Alpha isoform	Hydroxyapatite crystals	Microsphere scaffolds	Promote cell adhesion *in vivo* bone healing	Bone	167
		Alkaline phosphatase	Mineralized nanopaper	Enhance mechanical properties homogenous and spatial controlled mineralization	Bone	167
		Hydroxypeptide	Hydrogel/cryogel	Enhance mechanical properties	Cartilage	168
Chitosan	Alpha isoform		Films	Enhance mechanical properties. Promote cell adhesion and proliferation	Skin	169
	Alpha isoform		Films	Enhance mechanical properties to improve biological activities	Dermal tissue	170
	Shrimp alpha isoform		Porous microspheres	Enhance mechanical properties. Allow hESCs multi-lineage differentiation	3D cell culture	171
	Alpha isoform		Films	Enhance mechanical properties orientation of structural elements increase specific conductivity promote skin fibroblast adhesion, viability, and proliferation	Skin	172
Cellulose silk			Hydrogel	Induce hMSCs osteogenic differentiation	Bone	173
	Alpha Isoform	Hydroxyapatite	Hydrogel multilayer films or membrane	Enhance mechanical properties self-bonding function	Biomedical materials	174
Collagen	Shrimp alpha isoform		Microfibers composite	Enhance mechanical properties and induce cell alignment	Vascular muscle	174
	Squid pen beta isoform		Films	Complement collagen biocompatibility on fibroblast growth	Biomedical materials	175
Gelatin	Crab alpha isoform		Films	Enhance wound healing process	Biomedical materials	124
	Crab alpha isoform		Nanocomposite films	Enhance mechanical properties	Biomedical materials	176
	Crab alpha isoform		Hydrogels	Enhance mechanical properties	Tendons ligament	177
Gelatin methacrylate	Squid pen beta isoform		Hydrogels	Enhance mechanical properties	Vascular	178
Lignin	Alpha isoform		Microcapsule like system	Biocompatible anti-inflammatory activity	Skin	179

NIH-3T3 fibroblast cells were seeded on the substrates with varying groove sizes to evaluate their behavior for tissue engineering applications.[160] The organization of fibroblasts within the extracellular matrix (ECM) of native myocardial tissue is crucial for cell alignment, which influences the heart's electrical and mechanical properties.[161] However, fibroblast attachment to chitin substrates is low, potentially due to the lack of reactive species and positive charges on the chitin surface.[162] To improve cell attachment, chitin substrates can be partially deacetylated and coated with fibronectin, an ECM protein vital for growth, migration, cell adhesion, and differentiation.[163] Deacetylation to 30% enhances crystallinity and intramolecular hydrogen bonding from the remaining acetyl groups.[164]

Post-deacetylation, cells spread and covered the entire film, aligning along the micropattern's major axis on the glass-supported transparent chitin substrates (Fig. 19d).[160] The optical transparency of these substrates also suggests potential applications in retinal regeneration. The biocompatibility, nontoxicity, cytocompatibility, tunable biodegradability, and mechanical strength of ChNFs make them highly promising for regenerative medicine and tissue engineering.

ChNFs in biologically active matrices and TE applications are given in Table 3.

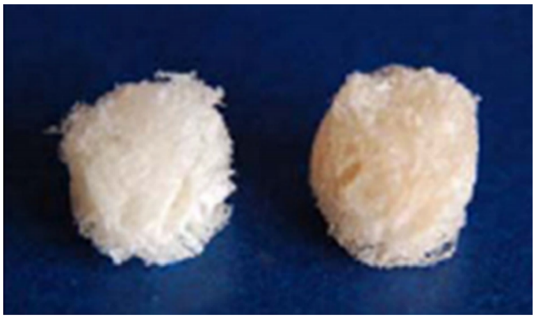

Fig. 20 Chitin scaffold (left) and chitin/nano Ag composite scaffold (right) are suitable for wound healing applications.[180]

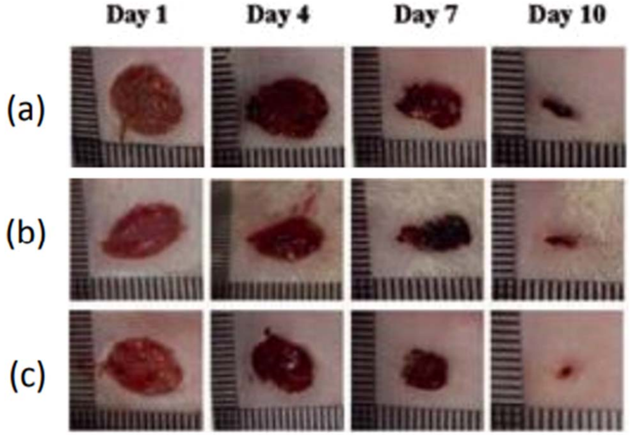

Fig. 21 Healings of the wound treated with three kinds of wound dressings at days 1, 4, 7, and 23 (a) gauze (negative control), (b) 30/70 CS-EDTA/PVA nanofiber scaffold and (c) commercial wound dressing (Sofra-tulle-register) (positive control).[183]

5.3 Wound dressing

Chitin-based membranes exhibit properties conducive to wound dressing applications, including good biocompatibility, high durability, low toxicity, antibacterial activity, and liquid adsorption. These properties can be further enhanced by incorporating polymers such as alginate, hyaluronic acid, poly(vinyl alcohol), α-poly(glutamic acid), polyethylene glycol diacrylate, and 2-hydroxyethyl methacrylate (Fig. 20).[180]

Studies have shown that α-chitin/nanosilver and β-chitin/nanosilver composite scaffolds exhibit antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, suggesting their potential as wound dressings.[181] Further, β-chitin-based composites have demonstrated favorable antibacterial, swelling, cell attachment, blood clotting, and cytotoxicity properties, supporting their suitability for wound dressing applications. Research also indicates that modified ChNFs derived from crab shells can improve clinical symptoms and control ulcerative colitis.[182]

Electrospinning has been employed to fabricate chitosan (CS) blended with ethylene diamine tetraacetic acid (EDTA) and polyvinyl alcohol (PVA) (CS-EDTA/PVA) nanofiber scaffolds. These scaffolds exhibit good antibacterial activity and promote wound healing (Fig. 21).[183]

A comparison between electrospun ChNFs and commercial chitin microfibers (ChMs) revealed that ChNFs, with an average diameter of 163 nm, facilitated superior cell attachment and spreading of normal human keratinocytes and fibroblasts compared to ChM (average diameter: 8.77 μm). ChNFs also exhibited a faster degradation rate. The high surface-to-volume ratios and three-dimensionality of ChNFs contribute to their potential for skin regeneration and wound healing applications.[38,184]

5.4 Drug delivery system and cancer diagnosis

Chitosan, a biocompatible biopolymer, has garnered extensive attention for its diverse applications in drug delivery, tissue engineering, and more. In cancer therapy, chitosan-based nanoparticles demonstrate promising capabilities for targeted drug delivery, minimizing side effects and enhancing therapeutic efficacy. These nanoparticles can either passively accumulate at tumor sites *via* the Enhanced Permeability and Retention (EPR) effect or target tumor cells using ligands specific to tumor receptors.[184,185] The nano chitin-fiber drug system has a higher specific surface area, shorter diffusion channels, and a greater release rate than bulk materials. Geetha *et al.*[186] investigated amorphous chitin nanoparticles (AC-NPs) loaded with curcumin (CUR), docetaxel (DOC), and 5-fluorouracil (5-FU). They found entrapment efficiencies of 98.1% for CUR-AC-NPs, 77.2% for DOC-AC-NPs, and 47.12% for 5-FU-AC-NPs. CUR-AC-NPs demonstrated superior drug uptake and increased cell death in gastric adenocarcinoma (AGS) cells, making them the most effective carrier among the studied AC-NPs.

Controlled-release drug delivery systems, enabled by chitosan, offer heightened safety and reliability in cancer treatment.[185] This technology facilitates a predictable release of therapeutic agents, reducing adverse effects while maximizing efficacy. For instance, chitin nanogels (ChNGs), prepared through controlled regeneration techniques, have exhibited increased swelling and biodegradability compared to chitin.[187,188] These ChNGs, combined with rhodamine-123 dye, demonstrate sound cellular localization without harming cells, suggesting their potential in drug delivery and tissue engineering. Moreover, carboxymethyl chitin (CM-chitin), a pH-sensitive derivative of chitin, can function as a hydrophilic matrix for controlled drug release. Recent studies have shown that CM-chitin releases aspirin at a slower rate in simulated gastric fluid than simulated intestinal fluid, indicating its potential for drug delivery applications, although further research is necessary to ensure safety and clinical viability.[187,188] The ChNGs have also been investigated for the delivery of anticancer drugs. Doxorubicin-loaded ChNGs, for example, have demonstrated biodegradability, biocompatibility, and toxicity toward various cancer cells, making them suitable for treating prostate, breast, lung, and liver cancer.[189] Chitin oligomers have shown potential as tumor growth inhibitors in mouse models, suggesting host-mediated effects.[190]

ChNFs, incorporated with anticancer agents like ellagic acid, have been explored for breast cancer treatment. Modified injectable hydrogels containing ChNFs have shown promise in anti-tumor drug delivery. ChNFs also find applications in bone regenerative engineering, a vital field in orthopedics for repairing damaged bones. Additionally, ChNFs synthesized

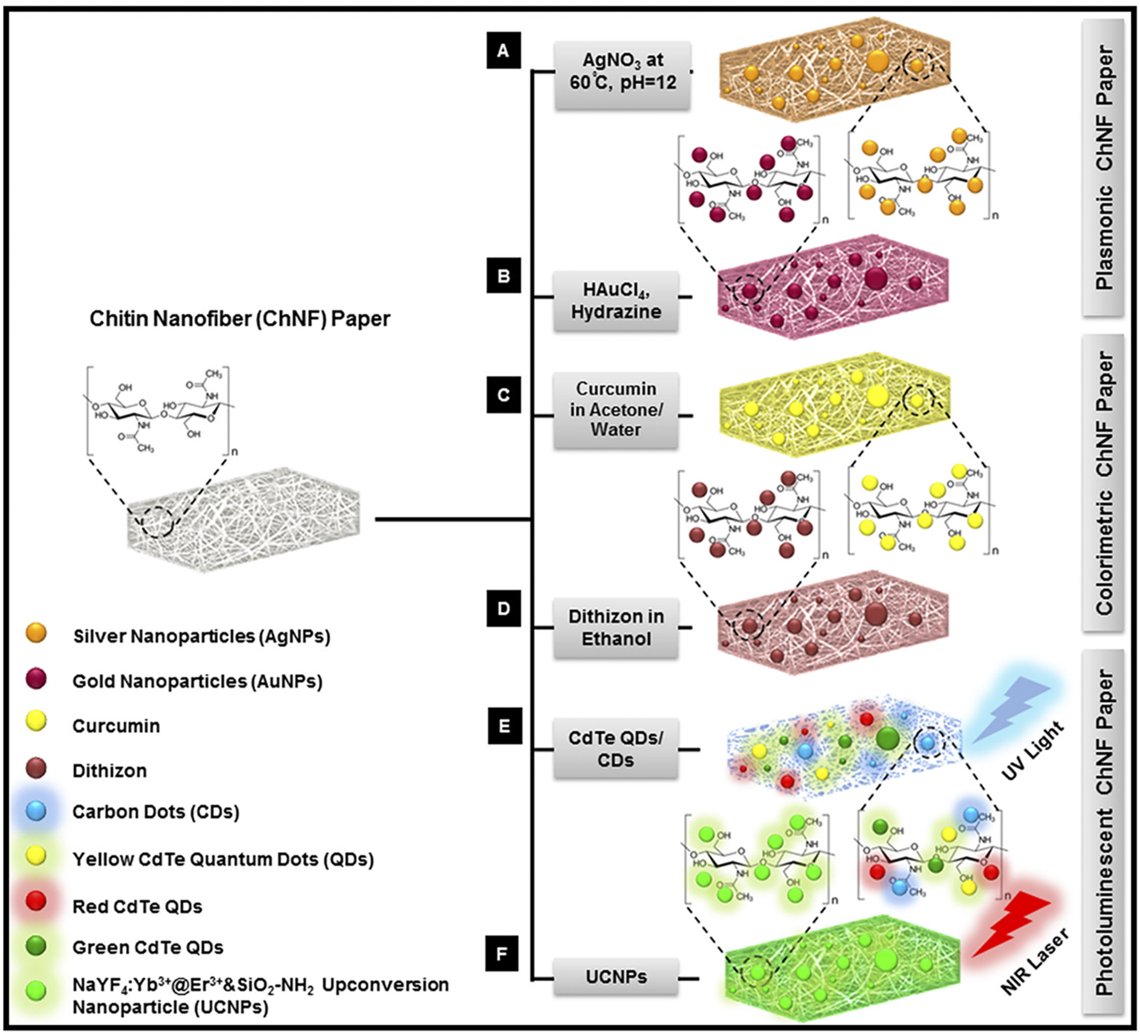

Fig. 22 Schematic representation of the fabrication of various ChNF paper-based nanocomposites: (A and B) *Plasmonic nanocomposites*: (A) silver nanoparticle-ChNF paper (AgNPs-ChNF paper); (B) gold nanoparticle-ChNF paper (AuNPs-ChNF paper). (C and D) *Colorimetric nanocomposites*: (C) curcumin-ChNF paper (Cur-ChNF paper); (D) Dithizone-ChNF paper (DTZ-ChNF paper). (E and F) *Photoluminescent nanocomposites*: (E) CdTe@ZnS quantum dot-ChNF paper (QDs-ChNF paper) and carbon dot-ChNF paper (CDs-ChNF paper); (F) aminosilica-coated $NaYF4:Yb^{3+}@Er^{3+}$ upconversion nanoparticle-ChNF paper (UCNPs-ChNF paper).[112]

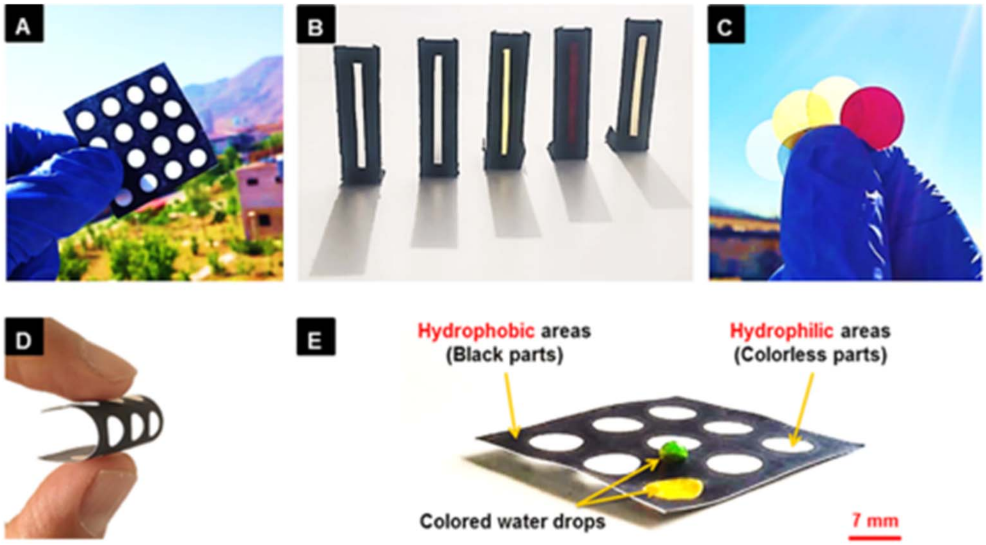

Fig. 23 Pictures of the fabricated ChNF paper-based sensing platforms with (A) 2D multi-wall, (B) 2D cuvette, and (C) spot patterns. (D) Picture showing the flexibility of the fabricated ChNF paper-based sensing platform. (E) Picture of the fabricated ChNF paper-based sensing platform.[112]

with UV-absorbing chromophores, such as urocanic acid, have demonstrated a protective effect against UV radiation-induced damage in mice. ChNFs have significant cytotoxic effects on various cancer cells while exhibiting low toxicity to non-cancer cells and can induce apoptosis.[191] Solairaj *et al.*[192] evaluated the toxicity of chitin nanoparticles (CNP), silver nanoparticles (AgNP), copper nanoparticles (CuNP), and their nanocomposites on MCF-7 breast cancer cells. They found that CNP combined with metal nanocomposites showed more significant cytotoxicity than AgNP or CuNP alone, with CNP/AgNP notably inducing apoptosis. Additionally, the toxicity of AgNP and CuNP to non-cancerous HEK-293T cells decreased with CNP, indicating higher activity against cancer cells.

Chitosan nanocomposites effectively retain and protect anticancer drugs like doxorubicin and dexamethasone, increasing their therapeutic efficacy. Chitosan-coated nanoparticles, including those loaded with doxorubicin, exhibit potent antitumor activity against various cancers, including ovarian cancer.[184] Furthermore, pH-sensitive polymers like chitosan facilitate controlled drug release in tumor tissues due to their responsiveness to the acidic tumor microenvironment.

Chitosan's inherent properties, such as biodegradability and pH sensitivity, make it an auspicious material for drug delivery systems. Understanding the molecular interactions between chitosan and drugs like doxorubicin is critical for optimizing drug delivery efficiency. Computer simulations, such as molecular dynamics, offer valuable insights into these atomic-level interactions, aiding in developing effective chitosan-based drug delivery.[193]

5.5 Biosensing applications

ChNFs possess numerous advantageous properties, including biodegradability, biocompatibility, commercial availability,

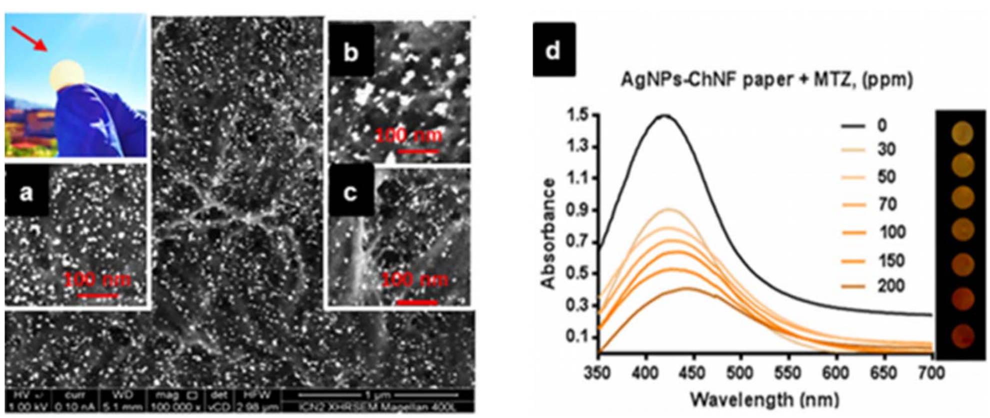

Fig. 24 Scanning electron micrographs of the fabricated AgNPs-ChNF paper: (a) *in situ* synthesized AgNPs-ChNF; (b) AgNPs-ChNF in the presence of 100 ppm MTZ; (c) AgNPs-ChNF paper in the presence of 3 ppm CN^-. The inset image (upper left) shows a digital photograph of the fabricated AgNPs-ChNF paper, and (d) displays the AgNPs-ChNF paper upon the addition of MTZ (0–200 ppm).[112]

affordability, abundance, flexibility, transparency, and remarkable mechanical and physicochemical attributes. Leveraging these beneficial features, researchers[112] have developed transparent, flexible, biocompatible, lightweight, and efficient optical sensing bioplatforms by embedding various plasmonic nanoparticles (NPs), such as silver and gold NPs, within ChNF paper. ChNFs can be derived from the abundant and cost-effective raw material of shrimp shells. In contrast, the production of bacterial cellulose (BC) nano paper, also employed in biosensing, is both time-consuming and expensive, whereas ChNF production is rapid, straightforward, and inexpensive (Fig. 22).[112]

The fabrication, sensing mechanisms, and characterization of various ChNF paper-based nanocomposites have been investigated.[112] Techniques such as UV-visible spectroscopy (UV-vis), field emission scanning electron microscopy (FE-SEM), energy-dispersive X-ray spectroscopy (EDX), and thermogravimetric analysis (TGA) have demonstrated the creation of transparent, flexible, biocompatible, compact, portable, and efficient ChNF paper-based optical sensing bioplatforms. These platforms align with the World Health Organization's (WHO) ASSURED criteria for ideal diagnostic devices, emphasizing sensitivity, specificity, user-friendliness, affordability, rapidity, robustness, equipment-free operation, and deliverability to end-users (Fig. 23 and 24).

Chitin, a polymer of *N*-acetyl glucosamine prevalent in fungal cell walls, insect exoskeletons, and other natural structures, serves as the precursor for chitosan. With its enhanced solubility and hydrogel-forming properties, Chitosan is more extensively utilized in biosensor applications.[194] Notably, the use of tyrosinase as a model enzyme immobilized on chitosan has demonstrated the creation of a sensitive phenol biosensor with a wide dynamic range, highlighting the adaptability of this approach for various sensing applications. Among bio-based polymers, chitin is the second most abundant natural polymer after cellulose. With an annual biosynthesis of approximately 10^{10}–10^{11} tons, this natural amino-polysaccharide, composed of β-(1,4)-2-acetamido-2-deoxy-D-glucose, is primarily found in the exoskeletons of marine shrimp and crabs, insects, fungi, and yeasts. Chitin has garnered significant attention in various fields due to its inherent advantages over conventional polymeric materials, including low cost, abundance, non-toxicity, renewability, biodegradability, sustainability, and biocompatibility.[112]

A highly sensitive and selective label-free electrochemical immunosensor has been successfully developed to detect prostate-specific antigen (PSA). This sensor utilizes a composite of chitosan, graphene, ionic liquid, and ferrocene (CS-GR-IL-Fc) drop-cast onto a screen-printed carbon electrode (SPCE) and subsequently frozen to generate a 3D porous cryogel layer (CS-GR-IL-Fc cry). The cryogel is further decorated with gold nanoparticles (AuNPs).[195] Additionally, glucose-treated rGO-activated carbon (rGO/AC) composites have enabled the detection of glucose within a range of 0.002 to 10 mM, exhibiting a sensitivity of 61.06 A mM cm^{-2}, a response time of 4 seconds and a low detection limit of 2 μM, further highlighting the potential of these materials in biosensor applications.[196]

5.6 Water treatment

Environmental protection has become a global priority, driving industries to seek cleaner technologies. Chitin and its derivatives, like hydroxyl methyl chitin, demonstrate potential in wastewater treatment due to their low cost, non-toxicity, and ability to bind pollutants.[185] Chitin's heavy metal chelating capacity has been demonstrated for metals including copper, iron, nickel, chromium, mercury, lead, zinc, cadmium, silver, and cobalt, with the strongest binding affinity observed for mercury and the weakest for cobalt.[197]

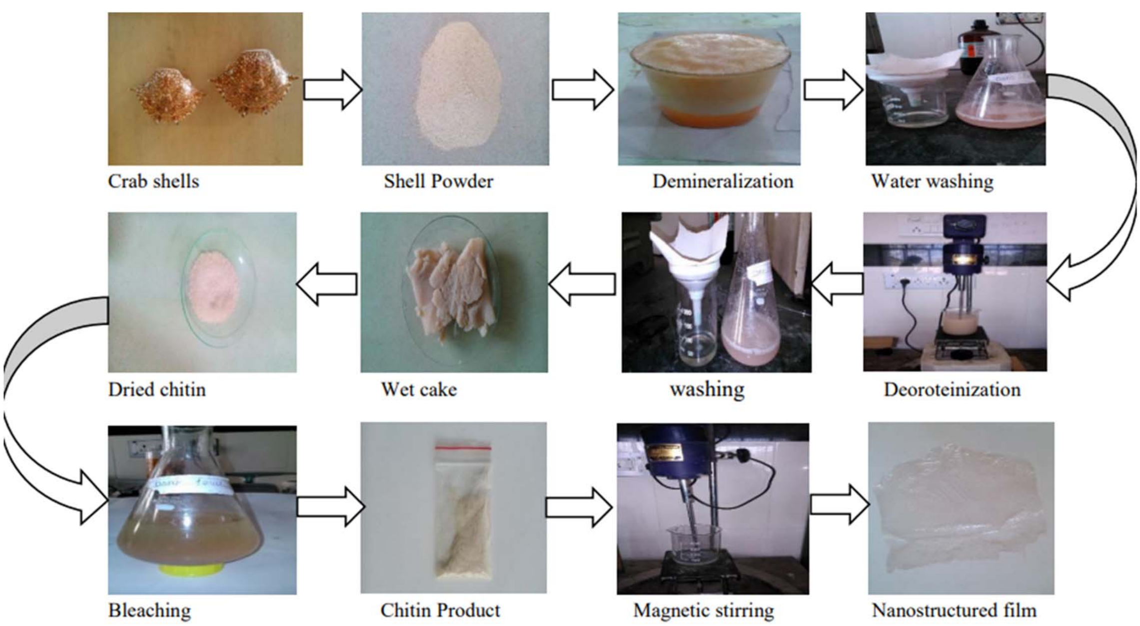

Fig. 25 Photographic presentation of nanostructured film production from crab shells.[59]

Biocomposites incorporating chitin with nano-hydroxyapatite (n-HAp) have effectively removed Fe(III) from aqueous solutions. The Fe(III) sorption capacity of n-HAp/chitin biocomposites (5800 mg kg^{-1}) surpassed that of n-HAp alone (4238 mg kg^{-1}), following a Langmuir isotherm and characterized as endothermic and spontaneous, suggesting applicability in water treatment. Similar experiments demonstrated the sorbents' selectivity for metal ions in the order Fe(III) < Cu(II) < Cr(VI).[198,199]

In the context of mining-influenced water (MIW) with its high concentration of toxic metals, both Chitorem SC-20 (raw crushed crab shell) and Chitorem SC-80 (chitin polymer) have been investigated. SC-20 effectively removed iron, lead, and zinc and partially removed copper, cobalt, and manganese. SC-80 partially removed cobalt, manganese, lead, and cadmium without precipitation.[200] Chitin/cellulose composite membranes, leveraging their microporous structure, have efficiently removed heavy metal ions like mercury, copper, and lead. The adsorption capacity followed the $Hg^{2+} > Pb^{2+} > Cu^{2+}$, increasing with higher chitin content. These membranes offer a "green" approach to wastewater treatment due to their regenerability.[201] Chitin hydrogels also exhibit promise in wastewater treatment due to their microporous structure, large surface area, and affinity for dye adsorption.[202]

Chitin and chitosan nanoparticles have numerous advantages in water filtration, including biodegradability, biocompatibility, and flexibility. Their performance is further enhanced when incorporated as nanofillers in biopolymer-based nanocomposites, offering improved surface area, mechanical stability, and customizable functionality. The unique properties of biopolymers enable the effective removal of heavy metals, organic pollutants, and microorganisms from water, making them valuable additions to membrane technologies and expanding their potential applications in water purification.[203]

5.7 Nanostructured film

The successful preparation of nanostructured films from chitin underscores its potential for creating value-added products. These films, being biodegradable and biocompatible, hold promise for applications as biomedical interfaces visually represent the nanostructured film production process from crab shells (Fig. 25).

ChNFs, with sizes ranging from 12 to 30 nm, were synthesized from chitin powder using a steam explosion technique followed by mild hydrolysis with oxalic acid. The CHNFs were then incorporated into natural rubber (NR) latex to create NR/CB/CHNF composites. It involved the uniform dispersion of ChNFs within the latex, followed by drying and mixing with carbon black (CB) using a two-roll mill. Remarkably, at a CHNF loading of 1 phr (parts per hundred rubber), the NR/CB/CHNF composite exhibited a substantial increase in mechanical properties compared to neat NR. Tensile strength improved by approximately 47%, and tear strength saw an even more significant enhancement of 160%. Furthermore, dynamic mechanical analysis revealed a 50% reduction in loss tangent (tan δ) at 60 °C for the NR/CB/CHNF 1.0 composite compared to the NR/CB50 composite. This study successfully demonstrated the development of an innovative and eco-friendly tire tread formulation, contributing to advancing a circular economy and sustainable practices.[204,205]

5.8 Food and nutrition

Chitin and its derivatives find extensive applications in the food industry, encompassing the creation of value-added food products, preservation against microbial spoilage, waste recovery from food processing, development of biodegradable films, water purification, acid removal and clarification of fruit juices, and color stabilization. They also serve as food additives, thickening agents, emulsifiers, and natural flavor extenders. Chitin's ability to control moisture transfer, antioxidant release, heat transfer, respiration rate, and enzymatic browning in fruits further expands its utility in edible film production. Research suggests that chitin oligomers' physiological activities and functional properties are influenced by their degree of polymerization (DP), with high DP oligomers exhibiting greater functionality than low DP oligomers.[206] These characteristics make them promising candidates for developing healthcare products, biopesticides, food, and additives.[207] ChNFs also have applications in the sugar industry for adsorbing melanoidins, complex biopolymers of amino-carbonyl compounds, from sugar syrup.[208]

Recent advancements include the development of chitin/chitosan whisker rectorite ternary films, demonstrating excellent water resistance and anti-bacterial properties, making them highly suitable for food packaging applications.[209] Studies on kelp grouper and *E. bruneus* have shown that diets enriched with chitin and chitosan can increase phagocytic activity, complement activity, red blood cell count, hematocrit, hemoglobin levels, and white blood cell count.[210] Additionally, dietary chitin supplements (5%) have been shown to modulate gut bacteria, including *Bacillus thuringiensis*, which exhibits bactericidal properties against fish pathogens, potentially offering a means to enhance disease resistance in fish.[211] Carboxymethyl chitin (CM-chitin) has also demonstrated anti-obesity and anti-adipogenic effects, assessed by measuring lipid accumulation.[212] These findings collectively highlight the significant potential of chitin-based materials in developing and processing novel food products.

ChNFs hold promise as antibacterial nanocomposite materials due to their inherent biocompatibility, organic nature, amino-containing macromolecular structure, and nano-size effects. In a recent study, molybdenum disulfide quantum dots (MoS2QDs) were successfully anchored onto partially dChNFs through aqueous reactions. The resulting MoS2QDs/dChNF nanocomposite demonstrated remarkable antibacterial activity against *Escherichia coli* under various conditions. At a concentration of 200 μg mL^{-1}, the survival rates of bacteria were significantly reduced compared to DEChN alone, particularly under near-neutral conditions (pH ≈ 6) with a bacteriostatic rate exceeding 90%. In contrast, MoS2QDs/TOCN (prepared with TEMPO-oxidized cellulose nanofibers) exhibited no apparent antibacterial activity, underscoring the crucial role of DEChN and its amino groups. The MoS2QDs/DEChN composite film shows potential for preserving meat by delaying spoilage.[213]

5.9 Cosmetic and toiletries

Chitin and its primary derivative, chitosan, hold promise in cosmetics and toiletries due to their unique fungistatic, fungicidal, and solubility properties in organic acids. Their applications span hair, oral, and skin care.[214] Notably, chitin and chitosan's properties make them ideal for treating acne, maintaining skin moisture, and enhancing hair suppleness and skin tone. They are found in various products like creams, lotions, permanent waving lotions, chewing gums, nail enamel, foundation, eye shadow, lipstick, cleansing materials, toothpaste, and bath agents. Certain derivatives also serve as nail lacquers.[16,215] In oral care, they are found to be used as dental fillers, false teeth cleaners, and agents that prevent plaque formation and tooth damage.[216]

Derived from insects, chitin and its derivatives are gaining traction in cosmetics for their biocompatibility and sustainability. They offer antioxidant and antimicrobial benefits, proving effective in diverse cosmetic and cosmeceutical applications.[217] Developing sustainable and innovative hair products can address the growing consumer demand for natural and eco-friendly cosmetics catering to skin and environmental health. Several abundant biopolymers, like chitin, chitosan, and lignin, exhibit specific functionalities (antimicrobial, antioxidant, anti-inflammatory, *etc.*). They can be combined in nanostructured tissues, powders, and coatings to create advanced cosmeceuticals with potential applications in other sectors, such as biomedical, personal care, and packaging. The cosmetics and wellness market is projected to grow significantly in the coming years. This trend, alongside advancements in nanobiotechnology, suggests the need for a shift towards a circular economy. This model prioritizes redesigning, reducing, recycling, and reusing products, promoting sustainability and responsible consumption.[218]

5.10 Agriculture

Chitin and its derivatives exhibit various properties, such as bactericidal and fungicidal activity, making them attractive

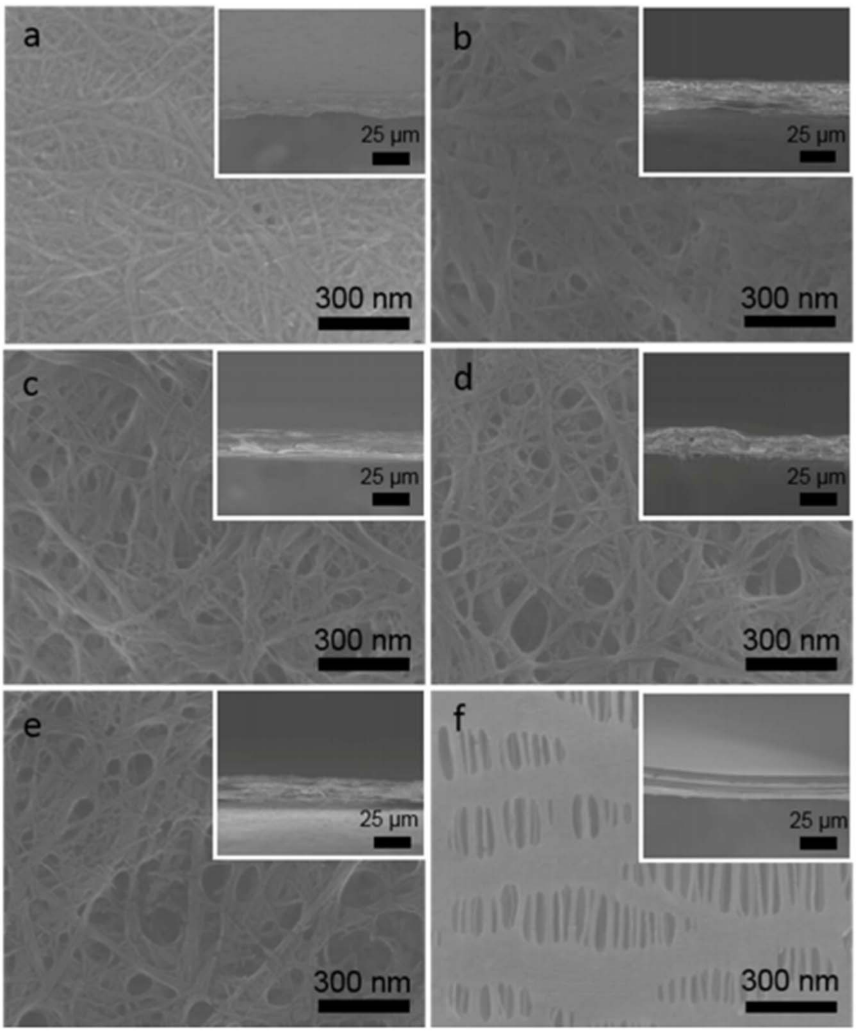

Fig. 26 Microstructural characterization of the CNM separators fabricated by the ChNFs from prawn shells and commercialized PP separator. (a–e) SEM images of CNM separators with various amounts of SDCA in ChNF suspension (0, 20 wt%, 30 wt%, 40 wt%, and 50 wt%, respectively) and corresponding cross-sectional SEM images (Insets). (f) The SEM image of the commercialized PP separator and corresponding cross-section image (Inset).[226]

candidates for agricultural applications. They have been used to assess mold contamination in agricultural products[215] and have shown potential to enhance plant growth and defense mechanisms. For instance, chitin-treated seeds exhibit accelerated growth due to decreased insect and fungal penetration.[219] Chitin oligosaccharides, derived from chitin/chitosan hydrolysis, can act as antioxidants, antimicrobials, and biofertilizers.[218] Additionally, chitin functions as a safe elicitor in controlled peanut cultures, inducing the production of *trans*-resveratrol and *trans*-piceatannol.[220] Chitin and its derivatives can also trigger defense responses in various plant species by inducing fungal microbe-associated molecular patterns.[221] Chitin and its derivatives find applications as fertilizers, soil conditioners, plant disease control agents, antitranspirants, natural product retardants, and seed coatings. They bolster natural plant defenses and act as plant growth regulators, growth stimulants, anti-stress agents, and elicitors for secondary metabolite production. While numerous reviews exist on chitosan in agriculture, this mini-review focuses specifically on the agricultural applications of chitin, not chitosan.[222]

5.11 Energy storage devices

Renewable and green energy sources like tidal, solar, geothermal, wind, and biofuels are increasingly prominent in the scientific community. These sources drive the rapid development of advanced energy storage systems with high energy densities.[223] As the demand for reliable electricity storage grows, advanced devices like fuel cells, supercapacitors, and separators for Li/Na-ion batteries are gaining importance. To ensure the sustainability of renewable energy on a large scale, developing low-cost, eco-efficient, and high-energy-density Li/Na-ion batteries is crucial.[224] Separators are critical in these batteries, impacting their safety, performance, and sustainability.[225]

Currently, commercial separators are made from polyolefins like polyethylene and polypropylene. While these materials offer advantages like high ionic conductivity and chemical/electrochemical stability, they have limitations, including low thermal stability and weak mechanical strength. These weaknesses can lead to high-temperature safety issues, potentially causing battery ignition or explosion. Moreover, their production from petrochemicals raises environmental concerns. ChNFs derived from prawn and crab shells present a potential alternative separator material. These nanofibers possess good mechanical strength, thermal stability, and sustainability.[226] Chitin is a naturally abundant resource with annual biosynthesis estimated at 10^{10} to 10^{11} tons [226] sufficient for large-scale separator production for Li/Na-ion batteries (Fig. 26).[226] Studies have shown that ChNF separators exhibit electrochemical performance comparable or superior to commercial polypropylene separators in $LiFePO_4$/Li and $Na_3V_2(PO_4)_3$/Na half cells.[226] Moreover, ChNFs can be used to produce low-cost, nitrogen-doped porous carbon electrodes with large surface areas and open hierarchical porous nanostructures, serving as a conductive substrate for zeolitic imidazolate frameworks (ZIF-8) derived porous carbon.[227] Nitrogen hierarchical porous carbon (N-HPC) electrodes derived from ChNC have demonstrated excellent capacitive performance in supercapacitors due to their high specific surface area, nanostructures, rich surface functional groups, and nitrogen content covalently bound with carbon atoms (Fig. 27).[227] These findings suggest that ChNF is a promising material that can contribute significantly to the future sustainable development of energy storage devices. Piezoelectric ChNFs are a new type of biodegradable sensor material. Chitin polymers dissolve with the chitinase enzyme in eight days without releasing toxic substances, making them promising for environmentally friendly piezoelectric materials. Hoque *et al.*[228] reported two high-performance piezoelectric nanogenerators (PENG) based on ChNFs: a pure ChNFs PENG

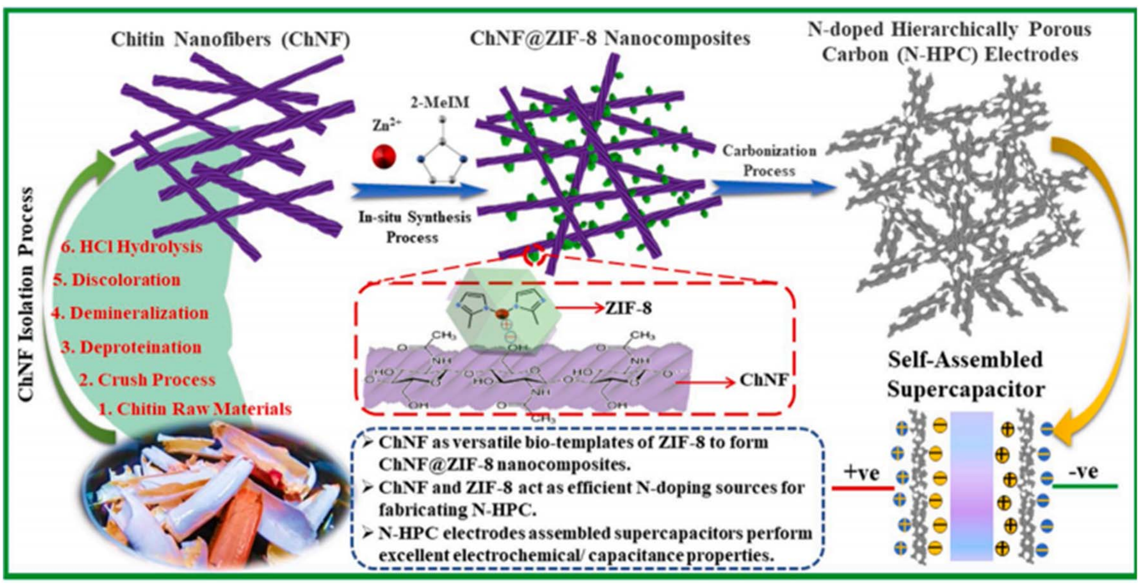

Fig. 27 Schematic illustration of the preparation of N-doped hierarchically porous carbon electrodes for high-performance supercapacitors.[227]

(CPENG) and a composite ChNFs/PVDF PENG (PCPENG). Both devices effectively conduct electricity from mechanical energy sources, including light touch and sonication, and have a short capacitance charging time, making them viable alternative energy sources for portable medical equipment. Chen *et al.*[229] developed a highly conductive film made of ChNFs and multi-walled carbon nanotubes (MWCNT) for foldable electronic devices. After hydrogel treatment, the ChNFs/MWCNT gel film became denser and exhibited nearly double the conductivity compared to the untreated film—9.3 S cm^{-1} for the gel film *versus* 4.7 S cm^{-1} for the original. This enhanced conductivity is promising for applications in electrodes or collectors.

While chitin- and chitosan-derived HCs have similar *d*-spacings and crystallite sizes, their pyrolysis results in different carbon structures. Chitin yields micro–mesoporous carbon with a high specific surface area, while chitosan produces nonporous carbon. Despite this, both materials initially exhibit a comparable specific capacity of 280 mA h g^{-1} (at C/10 rate). However, their electrochemical performance diverges upon prolonged cycling at higher rates. Inorganic contaminants in chitosan-derived HC may hinder sodium ion diffusion, slowing electrochemical reactions and leading to polarization buildup. Optimizing chitosan-derived HC through acid treatment can unblock micropores, increase carbon content, and enhance the active surface area, thus mitigating capacity fading.[230]

5.12 Other relevant applications

Novel research has explored the potential of various chitin nanowhiskers/nanofibers, including TEMPO-oxidized α-chitin nanowhisker, HCl-hydrolyzed chitin nanowhisker, partially deacetylated α-chitin nanowhisker/nanofiber mixture, and squid pen β-ChNFs. These materials have demonstrated promising oxygen barrier properties, suggesting their suitability for biocompatible and biodegradable films in various applications.[107] Furthermore, chitin-based molecularly imprinted polymers (MIPs), such as cholesteryl chitin carbonate, have been developed using covalent and non-covalent imprinting techniques. These MIPs exhibit high binding capacity with cholesterol in non-polar solvents through hydrogen bonding, making them potential candidates for sensing, separation, and delivery applications.[231]

A facile method employing the TEMPO/$NaClO_2$/NaClO system has enabled the fabrication of zwitterionic ChNFs through deacetylation and oxidation. The ChNFs facilitate chitosan immobilization of anthocyanin and enhance the rheological properties and printability of chitosan/anthocyanin/ChNF-based smart inks. Screen-printed labels incorporating these inks have been successfully used as fish freshness indicators, providing a convenient and visual strategy for assessing fish quality. The use of degradable, edible, and environmentally friendly ingredients in these labels further underscores their appeal.[219]

Active films composed of chitosan, esterified ChNFs, and rose essential oil (REO) have been developed in food preservation. The combined effects of ChNFs and REO on chitosan films' structural and physicochemical properties were investigated. Results showed enhanced water resistance, mechanical properties, UV resistance, increased oxygen permeability, and antioxidant activity.[232] Zou *et al.*[233] prepared ChNFs using low-intensity ultrasound and subsequently developed antibacterial finishing agents. Cotton fabrics treated with ChNFs showed less color difference than untreated fabrics, achieving an antibacterial rate of 99%. The study suggests good potential for using ChNFs in the textile industry. Another study explored the use of chitosan and esterified ChNFs with varying proportions of scallion flower extract (SFE) to create active packaging for banana preservation. Incorporating CF improved barrier properties and mechanical strength, while SFE enhanced physical and biological activity. The CF-4% SFE film demonstrated superior oxygen barrier performance, antibacterial efficacy, and antioxidant activity. Fresh-cut bananas stored in CF-4% SFE exhibited reduced weight loss, starch deterioration, color alteration, and visual changes compared to those in polyethylene film, highlighting its potential as a sustainable alternative to conventional plastic packaging.[234]

Finally, sustainable ChNF-coatings have been investigated to extend the storage life of fresh cucumbers and inhibit bacterial growth.[235] The degree of acetylation and deacetylation time influenced the morphology and antibacterial properties of ChNFs. ChNFs deacetylated for 120 and 240 min, exhibiting a fibril-like structure, significantly reduced moisture loss and bacterial growth on cucumber surfaces,[236] demonstrating their potential for reducing food waste and dependence on petroleum-based packaging materials.

6 Challenges and future outlook

Although the synthesis of chitin and chitosan nanofibers from various sources, such as crab, shrimp, and mushrooms, utilizing top-down and bottom-up approaches poses challenges to environmental sustainability and cost-effectiveness, the principal advantage of ChNFs over cellulose and silk fibroin lies in their hydrophilic properties and sensitivity to environmental conditions. This unique characteristic enhances their potential applications in various fields, including biomedicine and material science, where tailored interactions with environmental factors are essential. Synthesis methods such as mechanical disintegration and acid hydrolysis are being investigated. However, their environmental impacts require thorough evaluation through life cycle assessments (LCAs). Energy-intensive mechanical disintegration processes require optimization. Acid hydrolysis requires the management and recycling of significant amounts of acid, which raises environmental concerns. Industrial production of nano chitin must meet the quality of lab-synthesized materials. Batch-to-batch variations should be minimized through standardized testing protocols. Differences in raw chitin sources, such as crustaceans and fungi, can impact production consistency. The low solubility of chitin and chitosan nanofibers at neutral pH and the variability of their antimicrobial effectiveness under different conditions highlight the need for further research.

By enhancing its solubility, boosting its antimicrobial activity, and deepening our understanding of its mechanisms, chitosan, and its derivatives could revolutionize fields such as sustainable agriculture, medical wound care, and food preservation. Nanochitin demonstrates considerable potential in the realms of bio-composites and high-barrier packaging films. However, the relationship between the dimensions of nanochitin and its material properties remains inadequately understood. Future investigations should focus on optimizing the reinforcement capabilities of nanochitin in nanocomposite formulations while elucidating the interplay between filler dimensions and matrix interactions. Besides, the application of techniques like TEMPO oxidation facilitates the introduction of zwitterionic properties to nano-chitin. This modification enhances its ability to interact effectively across diverse environments, making it particularly advantageous for various biomedical applications. The zwitterionic characteristics contribute to improved biocompatibility and reduced protein adsorption, thereby enhancing the functionality of nano-chitin in areas such as drug delivery, tissue engineering, and biosensing. Further research into the mechanisms of these interactions could expand the potential uses of nano-chitin in innovative biomedical solutions. Furthermore, the thermodynamic analysis of highly optimized ChNFs structure integrated with diverse nanomaterials, catalysts, and drugs can be studied in future research. This analysis aims to yield insights into the efficacy of ChNFs, thereby enhancing our understanding of their potential applications in cancer therapy and other biomedical domains. Such studies could provide binding affinity for targeted treatment methods and the effectiveness of drugs interacting with ChNFs.

Apart from this, the moisture sensitivity inherent to nanochitin poses significant challenges to its application. Strategies such as thermal annealing and the incorporation of superhydrophobic surfaces, particularly through SLIPS (Slippery Liquid-Infused Porous Surfaces) technology, may provide viable solutions. The prospective applications of self-assembled nanochitin structures span across various fields, including optical systems, load-bearing materials, environmental management, and electrochemical devices. Nonetheless, challenges of fabrication and consistency remain prevalent within the field.

In addition, the exploration of chitin-derived carbon materials for electrochemical and environmental applications, including energy storage and catalytic functions, is still in its primary phase. There is a need for further research aimed at optimizing these materials for targeted applications.

7 Conclusion

This comprehensive review examined the methods for preparing chitin and ChNFs, along with their diverse applications. Numerous preparation methods have been documented, with ChNFs extracted from various sources, including crab shells, prawn shells, shrimp shells, squid pens, and mushrooms, using techniques like grinding or high-pressure water jet disintegration. The resulting ChNFs exhibit fine, uniform structures ($\approx$10–20 nm in width) with a high aspect ratio. Unlike traditional chitin, which is insoluble and precipitates in water, ChNFs disperse homogeneously, facilitating handling and molding for various applications. This study explores their utilization in biomedical applications (tissue engineering, wound dressing, drug delivery systems, cancer diagnostics), biosensing, water treatment, food and nutrition, cosmetics and toiletries, agriculture, energy storage devices, and other fields. Chitin and its derivatives, owing to their nontoxicity, biocompatibility, and biodegradability, are attractive natural materials for biomedical applications. This review aims to raise awareness of the importance of chitin, the second most abundant natural polymer, and its derivatives by discussing various aspects, including biological properties and applications. Despite progress, the production and applications of chitin, chitosan, and ChNFs derived from crab shells, prawn shells, squid pens, and mushrooms remain limited, necessitating further research to address knowledge gaps. Moreover, most studies to date have been conducted *in vitro* or *in vivo*, highlighting the need for clinical investigations to establish the true potential of chitin and ChNFs in clinical practice.

Data availability

No new data were created or analyzed during this study. Data sharing is not applicable to this article.

Conflicts of interest

The authors declare no conflict of interest.

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