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Biopolymer nanofibrils: structure, modeling, preparation, and applications

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Abstract

Biopolymer nanofibrils exhibit exceptional mechanical properties with a unique combination of strength and toughness, while also presenting biological functions that interact with the surrounding environment. These features of biopolymer nanofibrils profit from their hierarchical structures that span angstrom to hundreds of nanometer scales. To maintain these unique structural features and to directly utilize these natural supramolecular assemblies, a variety of new methods have been developed to produce biopolymer nanofibrils. In particular, cellulose nanofibrils (CNFs), chitin nanofibrils (ChNFs), silk nanofibrils (SNFs) and collagen nanofibrils (CoNFs), as the four most abundant biopolymer nanofibrils on earth, have been the focus of research in recent years due to their renewable features, wide availability, low-cost, biocompatibility, and biodegradability. A series of top-down and bottom-up strategies have been accessed to exfoliate and regenerate these nanofibrils for versatile advanced applications. In this review, we first summarize the structures of biopolymer nanofibrils in nature and outline their related computational models with the aim of disclosing fundamental structure-property relationships in biological materials. Then, we discuss the underlying methods used for the preparation of CNFs, ChNFs, SNF and CoNFs, and discuss emerging applications for these biopolymer nanofibrils.

Graphical Abstract

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Keywords

biopolymers; nanofibrils; collagen; chitin; silk; cellulose

1. Introduction

Biopolymer nanofibrils are universal nanobuilding blocks in natural materials [1–4]. These materials include cellulose nanofibrils (CNFs) in plants and bacteria, chitin nanofibrils (ChNFs) in animals, silk nanofibrils (SNFs) in spider and silkworm cocoon silks, as well as collagen nanofibrils (CoNFs) in ligaments and the dermis of echinoderms, mammalian tendons, and bones. The detailed structure of CNFs [5], ChNFs [6], SNFs [7] and CoNFs [8] are sketched in Figure 1. These biopolymer nanofibrils are composed of different biopolymer molecules, *i.e.*, cellulose, chitin, silk fibroin, and collagen, and thus feature various chemical reactivities; however, they usually share common characteristics at higher structural levels. For example, in the cases of CNFs, ChNFs and SNFs, they were considered as semicrystalline polymer-like nanofibrils with highly-oriented nanocrystals embedded in the amorphous matrix [9, 10]. In most of the biological materials, these single nanofibrils are further bundled into highly-orientated microfibrils (also known as fibrils) or even fibril bundles [11]. These hierarchical mesostructures further endow biological materials with exceptional mechanical performance, such as the superior toughness of spider silk fiber (even several times that of steel and Kevlar fibers) [12, 13], excellent defect resistance of bones [14, 15] and exoskeletons of crustaceans [4], as well as the smart bucking adaptability of woods [16]. Accordingly, understanding the structural constructions of the nanofibrils in nature will help to disclose performance-optimization mechanisms in biological materials and also inspire new engineering material designs.

In addition, as four of the most abundant biopolymer nanomaterials in nature, CNFs, ChNFs, SNFs (especially those produced from *B. mori* silk fibers), and CoNFs have received a great deal of scientific and commercial attention in recent years due to their outstanding mechanical properties, wide-availability, sustainability, biocompatibility and biodegradability (for ChNFs, SNFs and CoNFs) [17–20]. A variety of bottom-up and top-down approaches have been developed to isolate and regenerate these biopolymer nanofibrils from biological materials, *e.g.*, woods, cotton, exoskeleton of crustacea, and *B. mori* silk fibers. The timeline of the progress in biopolymer nanofibrils is summarized in

Figure 2. In comparison with the conventional biopolymer materials produced from biopolymer solutions[21], where the biopolymers have been dissolved to the molecular scale, the biopolymer nanofibril-based materials maintained sophisticated meso-architectures found in natural material systems and thus endow the regenerated materials with new structures and properties that originated from the nanosize effects. For example, the biopolymer nanofibril-based materials are usually featured by the extracellular matrix-like textures, nanoporous structures, nanoconfinement effects and optical transparency. These unique traits provide new avenues for biopolymer material applications in a wide range of fields, including biomedicine, optics, energy and electronic devices and environment [18–20, 22].

In this review, we first introduce the structures and computational models involving biopolymer nanofibrils, including CNFs, ChNFs, SNFs and CoNFs. Then, we summarize experimental methods to produce these biopolymer nanofibrils and outline their applications. Finally, we discuss the broader challenges and directions for the future development of biopolymer nanofibrils for functional material designs and engineering. These emerging topics related to the biopolymer nanofibrils offer new insight to understand underlying material design principles in nature, while also providing guidelines for the preparation and utilization of these versatile biological nanomaterials in a range of new devices and functions.

2. The structures of biopolymer nanofibrils

Understanding the universality-diversity paradigm of biopolymer nanofibrils (for example, the universality in the hierarchical structure and the diversity in chemical composition) can help us gain insight into materials design principles in nature, and also can inspire new engineering material designs and fabrication. Accordingly, we first summarized the structures of biopolymer nanofibrils in biological materials.

2.1 CNFs

Nanocellulose is the most abundant biopolymer nanomaterial in nature [18], usually produced by plants, marine animals, algae, fungi, and amoeba. In most organisms, CNFs act as structural components in cell walls. Chemically, nanocellulose is composed of cellulose, a linear polymer of β 1,4-linked glucan chains (Figure 1) [18, 23]. Nanocellulose can be classified into cellulose nanocrystals (CNC) and CNFs according to their morphology. CNF refers to nanofibrils with long lengths and high aspect ratios, while the nanofibrils with high crystallinity and relative shorter lengths and lower aspect ratios are termed as CNC or cellulose nanowhiskers. The term cellulose nano-objects is sometimes used as well and can describe both CNFs and CNCs. Another kind of CNF is bacterial cellulose (BC), which refer to nanostructured cellulose produced by bacteria [24].

At the single nanofibril scale, CNFs consist of both crystalline and non-crystalline (amorphous) regions which are usually placed in alternate arrangements and stabilized by hydrogen bonds and van der Waals forces. In some species, the amorphous regions can encapsulate the crystalline parts [18, 23, 25]. There are four different crystalline states for cellulose, cellulose I, II, III, and IV, featuring differences in the location of hydrogen bonds

between and within strands. The native nanocrystal structure is defined as cellulose I, which has two allomorphs, *i.e.*, Ia and Ib. The Ia crystalline dominates in bacteria and algae, and Ib crystalline is rich in plants. Both allomorphs can be found from the same source and possibly in the same nanofibril. Ia cellulose crystals have a triclinic P1 space group, containing one cellulose chain in each unit cell with parallel chains of identical conformation. The unit cell parameters are $a=0.672$ nm, $b=0.596$ nm, $c=1.040$ nm, $\alpha=118.08^\circ$, $\beta=114.80^\circ$, $\gamma=80.375^\circ$ [26]. While Ib cellulose crystals have the monoclinic P21 space, with two non-identical chains arranged in a parallel manner [27]. The unit cell parameters in Ib cellulose crystals are $a=0.778$ nm, $b=0.820$ nm, $c=1.038$ nm, and $\gamma=96.5^\circ$. The major difference between these two crystal structures is the relative displacement of cellulose sheets along the (110) lattice plane in the triclinic structure and (200) lattice plane in the monoclinic structure, called “hydrogen-bonded” planes, in the chain axis direction. There is a relative displacement of $c/4$ between each subsequent hydrogen-bonded plane for Ia crystalline, while the displacement alternates between $\pm c/4$ through van der Waals interactions for Ib crystalline [18].

The basic fibrillar unit in CNF is the elementary fibril, which is a universal structural element of natural cellulose and exists in wood, bamboo, cotton, ramie, jute and bacteria [28]. Several structural models have been proposed for an elementary fibril. A hexagonal arrangement of 36 chains is thought to be an optimal model because the cellulose synthase rosette complex from which cellulose is synthesized in plants is believed to be composed of 36 cellulose synthase catalytic proteins. However, this model has been challenged by wide-angle X-ray scattering and small-angle neutron scattering, which suggested that 36 chains in an elementary fibril may be an overestimation. Computationally, simulated diffractograms for 18- and 24-chain models, with and without constant or regular shape, show a better fit to the experimental characterization than the 36-chain model [27]. The diameter of the elementary fibril is approximately 3.5 nm, and these individual elementary fibrils can further align and bond together into nanofibrils (<35 nm), and can be further bonded into fibrils (<1 μm) and fibers (10–50 μm) [18, 23, 28].

2.2 ChNFs

Chemically, chitin is a cellulose analog—a long-chain polymer of a (1,4)- β -*N*-acetylglucosamine, a derivative of glucose (Figure 1) [29]. The three crystalline forms of chitins are known as α -, β -, and γ -chitins. In α -chitin, all the molecular chains are arranged in an antiparallel mode with strong inter- and intra-molecular hydrogen bonds. β -chitin has a parallel chain packing mode and weak hydrogen bonding between intrasheets. α -chitin has a more tightly compacted structure than β -chitin. Along the b axis of the unit cell, α -chitin has inter-sheet hydrogen bonds, but in the same direction, no hydrogen bonds exist in β -chitin [29]. γ -Chitin is less characterized and is reported to form two antiparallel chains and one parallel chain, *i.e.*, a mixture (or intermediate form) of α - and β -chitins [30]. Yet, recently study revealed that γ -chitin is closer in structure to α -chitin than β -chitin [31]. Most natural chitins have the α -type crystal structure, such as chitins extracted from prawn, crab, shrimp, lobster shells and mushrooms, while β -type chitin is present in squid pens and tubeworms [32]. γ -Chitin exists as the rarest crystal structure among these polymorphs and is present in cocoon fibers of the *Pinus* beetle and the stomach of *Loligo*[30]. The diameter of ChNFs

varies in a range of 1.5–25 nm, depending on the source organism [33]. Typically, ChNFs in invertebrates have a diameter of 1.5 nm [34]. Like CNFs, ChNFs usually assemble into fibrils and then into meso- and macro-scale hierarchical structures [3]. For example, in the exoskeleton of arthropods, ChNFs (2–5 nm in diameter and approximately 300 nm in length) are confined by a ~1.5 nm thick sheath of proteins and are assembled into elongated fibrils with diameters of 50–350 nm that are embedded in a mineral-protein matrix. At higher mesoscales, the chitin-protein fibrils align as planar layers along their normal direction with helicoidal stacking; each layer of the fibers is rotated from the previous stacked layer by a constant angle, and the arrangement completes a full 180° rotation. These arrangements are known as helicoidal structures, twisted plywood or Bouligand structures [35]. Similar structures appear in chiral nematic liquid crystals (historically referred to as cholesteric).

2.3 SNFs

SNFs, as the most critical nano-elements in spider and silkworm cocoon silk fibers, present several common structural features in different species. For example, all single SNFs feature beaded topological structures that are connected by homogeneous nano-globules with the diameter fluctuation in the range of 3–5 nm. In both spider and silkworm cocoon silks, these nanofibrils are bundled into 20–200 nm thick fibrils (also termed microfibrils) and are further organized into silk fibroin filaments [11]. However, different silk fibers feature complexity and chemical variability at the primary structure (amino acid sequence) [36–38], which is traced back to the diversity of the different types of silk fibers related to function. It has been confirmed that more than 30,000 known species of spider and 113,000 species of *Lepidoptera* insect can produce silks [39]. The spider dragline silks (*Nephila clavipes* and *Araneus diadematus*), domestic silkworm (*Bombyx mori*) cocoon silk, wild silkworm (*Antheraea pernyi* and *Samia cynthia ricini*) cocoon silks are the most well-studied natural silk fibers.

At the primary structural level, these silks consist of highly repetitive core sequences with alternative hydrophilic and hydrophobic segments and flanked by the highly conserved N- and C-terminal domains [40–42]. The diversity of the amino acid sequences of the different silks come from their repetitive core sequences. For example, in the hydrophobic domains, the spider dragline silks are constructed by (Ala)_n (n=4–6) motifs, while the *B. mori* silk fibroin consists of (Gly-Ala-Gly-Ala-Gly-Ser)_n (n=1–11) [40]. In the hydrophilic motifs, both silks contain tyrosine-rich sequences.

In the silk glands, the silk fibroin proteins appear as aqueous micelles, which helps to stabilize and organize the proteins at high concentrations [43]. During the natural spinning process, the hydrophilic and hydrophobic domains are transformed into a random coil (and/or helical) and β -sheet structures, respectively [44–46]. Accordingly, the SNFs can be considered as semicrystalline polymer-like nanofibrils with highly-oriented antiparallel β -sheet nanocrystals embedded in the amorphous matrix (consisting of a random coil and/or helix structures) [9]. Different silk fibers also have different β -sheet sizes and content due to the differences in β -sheet formation capability decided by the length and percentage of hydrophobic sequences in the protein chains [47, 48]. Raman spectroscopy [49], ¹³C cross polarization magic-angle spinning nuclear magnetic resonance (CP/MAS NMR) [50, 51], X-

ray diffraction (XRD) [52, 53], and Fourier-transform infrared (FTIR) spectroscopy [47, 48] have been used to assess the β -sheet content of silk fibers. Although the absolute values generated from the various methods are different, the trend in β -sheet content in these silk fibers are the same, where the *B. mori* silk and spider dragline (major ampullate) silk have the highest and lowest β -sheet content, respectively, agreeing with the ordered fraction changes calculated from the amino acid sequences of these silk proteins [47].

2.4 CoNFs

Collagen is the most abundant protein in mammals, making up from 25% to 35% of the whole-body protein content [54]. So far, 28 types of collagen have been identified [55], among them, Type I, II, III, IV, and V are most common types and type I, II, III, V and XI are fibrillar structures [55]. Type I collagen is the major collagen found in bones, tendons, corneas, and ligaments 90% of all collagen in the body [54]. Type I collagen is a heterotrimer, where each triple helical strand consists of two $\alpha 1$ (I) chains and one $\alpha 2$ (II) chain. Type II collagen is a homotrimer composed of three $\alpha 1$ (II) chains that form fibrils in cartilage, vitreous and spinal cord [56]. Type III collagen is also a homotrimer that coexists with type I collagen in collagenous fibrils of extensible tissue [56]. For example, about 60% of type III collagen and 40% of type I collagen are found in blood vessels; skin fibrils contain 15% type III collagen and 85% type I collagen [57]. The unique characteristics of type III collagen are the disulfide bonds in the C-terminal domain of the three-helix which serve as the cross-linkers to connect and stabilize the three chains. Collagen types V and XI are all heterotrimers, which coexist with fibrils in types I, II, and III collagen [56].

Here, we discuss the most abundant type I collagen (Figure 3) [58]. It contains three α -polypeptide chains, each containing 1,014 amino acids and adds up to 3,042 amino acids for a well-folded molecule [59]. Each α -chain is composed of a N-terminus, a series of Gly-X-Y repeats and a C-terminus. In Gly-X-Y repeats, X and Y represent any amino acids but are usually proline and hydroxyproline (Figure 3A) [56]. The proline eliminates the free rotation of the angle ϕ , but slightly increases the rotation of the angle ω , increasing the rigidity of the chain segment (Figure 3A), while the hydroxyproline usually is 4-hydroxyproline or 5-hydroxyproline [60]. Interestingly, hydroxyproline and hydroxylysine are rarely identified in other proteins, and both are formed from enzymatic post-translational modification steps during collagen biosynthesis. Hydroxyproline in the triple-helical domain provides the binding sites for water molecules and plays a critical role in hydrogen bond formation and the stability of the triple-helical structure. In nature, all collagens are a left-handed α -helical structure [61]. The formation of this helical structure is mainly caused by the electrostatic repulsion between the proline at the X position and the 4-hydroxyproline at the Y position. In the α -helix chains, the amino acid residues in the side chains are all outward of the helix axis and help to form hydrogen bonds in the helical chain. These specific arrangements of the amino acids make the helical structure are highly stable [62].

The tertiary structures of different collagens vary widely, but they share one crucial characteristic structure, that is, the triple-helical structure, which consists of three left-handed helical polypeptide chains entangled with each other to form a right-handed triple-helical or “superhelical” collagen called tropocollagen [63]. The first triple-helix structure of

collagen was reported in 1955 [64, 65]. Further, the 10/3-helix model was established in 1961 to explain the triple-helical structure of native collagen [66]. The average relative molecular mass of tropocollagen is about 300 kDa, the length is approximately 300 nm, and the diameter is 1.5 nm (Figure 3B) [67]. The α -helix in tropocollagen contains 3.3 amino acid residues per period ($n = 3.3$) with a pitch of 0.96 nm. Along the central helix axis, the distance between adjacent residues is 0.29 nm with a rotation angle of 108° [67]. Therefore, the three strands of tropocollagen present an extended conformation which is challenging to stretch. The stability of these helix depends on the hydrogen bonds between polypeptide chains. Because the direction of rotation of the triple helix is opposite to the direction of rotation of the polypeptide chains, the structure is less prone to unwinding, giving high strength to match the physiological functions of collagen.

In fibrillar collagens, the tropocollagen subunits further spontaneously assemble into larger arrays with regularly staggered ends due to the presence of the alternant polar and non-polar domains of α -chain (Figure 3B). The collagen molecules are staggered to adjacent molecules by about 64 nm or 67 nm (a unit that is referred to as 'D' and changes depending upon the hydration state of the aggregate), while the vertical distance between adjacent molecules is around 40 nm. In each D-period repeat of the microfibril, there is a part containing five molecules in cross-section, called the "overlap" or dense region, and a part that is containing only four molecules called the "gap" or sparse regions [68] (Figure 3B and C). These overlap and gap regions are retained in CoNFs and further assemble into microfibrils. The triple helical tropocollagens in the microfibrils are arranged in a quasi-hexagonal packing pattern. In some hard tissues, such as bones, the CoNFs are highly mineralized and organized into fiber structures with diameters of $\sim 5 \mu\text{m}$ [8]. At the higher hierarchical level, for example, in the osteons and Haversian canals (two key structures of bones) [8], the fibers are arranged into a helicoidal structure with similar features to the cellulose nanofibrils in woods.

3. Computational Modeling of biopolymer nanofibrils

The advantageous mechanical performance of biopolymer nanofibrils not only lies in its fine control of the structure of the compositions at the nano-scale but also the delicate hierarchical structure that integrates the complexity of the system. A comprehensive understanding of the mechanical properties and synthesis strategy adopted by the natural structures requires systematic study across multi-scales. Computational modeling is an effective way to achieve this task because it can reveal the molecular information at the nanoscale, which is the underlying basis for the functions of biopolymer nanofibrils at the macroscale. Further, these are more difficult fundamental features to understand from experimental approaches. Thanks to the progress in experimental characterization and computer technology [69–74], in recent decades, atomistic models and force fields have rapidly developed for studying biopolymer nanofibrils [75–85]. In this section, we review the recent progress in biopolymer nanofibril simulations.

3.1 CNFs

Crystalline cellulose is the major component in wood and plays a critical role in determining the stiffness and strength of the wood. As mentioned, the glucose units are connected linearly through β -D-1–4 linkages to form long cellulose chains, and multiple chains stack together to form the crystals. Once the crystalline cellulose is stretched, the covalent bonds along the cellulose chain form and thus provide high stiffness and strength for the material. Various simulation studies have confirmed the high stiffness of the crystalline cellulose, which is above 100 GPa [86–90]. The strength along the direction of the cellulose chains is around 6 GPa by simulations using reactive force fields [88]. The high strength of cellulose is a result of the fact that the fibrils fail with the breaking of the covalent β -D-1–4 linkage. Another important feature that renders the high stiffness and strength of cellulose is the delicate network of hydrogen bonds (H-bonds) within the cellulose crystals. As shown in Figure 4A, the rich hydroxyl side groups in cellulose provide abundant donors and acceptors for the formation of intra-chain and inter-chain H-bonds. The intra-chain hydrogen bonds are along the direction of cellulose chains and provide extra stiffness to the crystal, although this contribution is minor compared to the covalent bonding. In the lateral direction, inter-chain H-bonds are the major contributor to the stiffness and strength [86, 88, 89]. Once the cellulose is loaded in the lateral direction, the failure of the crystal results in the separation of the cellulose chains, instead of the cleavage of covalent bonds as in the direction of cellulose chains. The different modes of deformation and failure lead to significantly different stiffness and strength along the lateral direction, which is 300–400 MPa according to computational study [88], and around one order of magnitude lower than that along the chain direction. In addition to the direct contribution to the stiffness and strength, the H-bond network also contributes indirectly to the mechanical properties by tightly bonding and stabilizing the ordered structure. In contrast to the disordered bending and torsion of the cellulose chains in the amorphous state [86, 91], the H-bonds enforce the cellulose chains to be straightened because bending is not energetically favorable, and the torsion is prohibited. Simulation studies have shown that the overall effect of H-bonds is not just a simple multiplication of the number of H-bonds but further amplified because of the cooperative effects between the H-bonds [92, 93]. By comparing the fracture energy of cellulose crystals with various thicknesses in the stacking direction, computational modeling revealed that the fracture energy was maximized above the thickness of ~6 nm, because of the reduced effects of the edge defects as the dimension increases [94]. The result explained the nano-dimensions of the CNFs found in nature.

In most biological materials, cellulose does not exist alone in nature but assembles with other types of organic material to form composites. For example, In the wood (the most abundant resources of cellulose), CNFs are wrapped in a soft matrix composed of hemicellulose and lignin. A comprehensive understanding of the mechanics of the wood should consider these components as integral to the cellulosic material functions. Molecular simulations have been performed to study the interaction between the hemicellulose/lignin molecules and the crystalline cellulose. The results show that hemicellulose chains mostly lay flat on the cellulose surface and chains are parallel [95, 96]. The modeling work also serves to construct assembly models of the overall wood that includes hemicellulose, lignin, and cellulose. The model of wood is constructed by assembling cellulose, hemicellulose, and

lignin in a sandwiched manner such that hemicellulose sticks on the cellulose surface and lignin resides in the middle region [97–99] (Figure 4B). The model could be further applied to study the material distributions within the secondary cell wall of wood or used for mechanical loading to assess the mechanical properties. As inter-fibril shear was applied on the model, the elastic and yield behavior of the material was observed, and the irreversible yield was attributed to the reorganization of the matrix molecules and also the sliding of matrix molecules along the CNFs resulting in breaking and reconstruction of the H-bond interactions [98]. While these studies only help to identify fundamental interactions at the molecular level, they lack the essential structural features of wood at the mesoscale, that is the microfibril angle. Coarse-graining of the atomistic modeling has been performed by matching the mechanical responses of the molecules [100, 101], where the scale of the model can reach hundreds of nanometers, to assess the mechanical properties of wood cell walls with the various microfibril angles [101]. As the microfibril angle increased, the stiff CNFs deviate from the direction of bearing the load and the material is softer. Instead, the sliding between matrix molecules and cellulose are more prominent.

3.2 ChNFs

Like crystalline cellulose, the glucosamine units in chitin are connected by a β -D-1-O-4 linkage to form long linear chains, and multiple chains form nano-crystals through the H-bond network and van der Waals interactions. Both the covalent bonds and H-bonds along the chain direction contribute to the high stiffness, which has been reported by various simulations to be around 80–100 GPa [102–104]. This value is lower than CNCs, partially because of the larger inter-chain spacing because of the presence of the acetyl groups. The significance of the acetyl group on improving the crystal strength along the lateral direction has been studied by simulations that compare the fracture of chitin with chitosan [105, 106]. The results show that the acetyl groups provide extra interactions and are responsible for the higher fracture energy and strength.

In nature, ChNFs associate with proteins and minerals to form nanocomposites. The interactions between these components provide the fundamentals for the overall mechanics of the composite materials. Simulation efforts have been applied to study the interaction between crystalline chitin and different proteins motifs to elucidate the effects of the secondary structure of a protein on the mechanical interactions (Figure 5) [102, 103, 107]. The results highlight the effects of the termini of proteins and the secondary structure on the interfacial strength between chitin and proteins, emphasizing the importance of modification of the interfaces during material synthesis [102]. Simulations of the composite model of the assembly of chitin and protein provide a deeper understanding of the molecular response of the composite under load. The length of the ChNF and the atomistic roughness of the chitin surface due to the acetyl groups are highlighted as essential for the mechanical properties of the composites [103]. Same with other biological nanocomposites, the study of the hierarchical structure of ChNF-based composites is necessary for a full understanding of structure-function. Multi-scale simulations by incorporating ab-initio calculations of crystalline chitin and retrieving the unknown properties of proteins have been proposed to explain the multi-scale mechanics of shrimp cuticles [104]. The model revealed the importance of the helicoidal structure on the reinforcement of the protein-bonded ChNFs

and improving the toughness of the composites. However, this strategy lacks the details of the molecular information that is fundamentally responsible for the mechanical response at the macroscale. Direct multiscale modeling would be a solution. Efforts have been applied to develop a coarse-grained model of ChNFs that reproduce relevant mechanical properties [108], to enable performing simulations on ChNFs of several hundreds of nanometers in length, which is more realistic. To the best of knowledge, there has been no coarse-grained modeling of chitin-protein composites, an important future research direction.

3.3 SNFs

Although SNFs have been imaged using atomic force microscopy (AFM) and electronic microscopy techniques as early as the 1950s [109], their structural details, as well as their role in the determination of the mechanical properties of silk fibers, have long been overlooked. This situation is due to the limited methods available until recently to extract them from native silk fibers without destroying their morphologies and physical properties. Previously, silk fibers were usually regarded as homogenous semicrystalline polymer fibers, and their mechanical properties were directly associated with the secondary structures of the silk fibroin. For example, the rubber-like network model [9] considers silk fibers as a two-phase cross-linked network, where β -sheets play a role as crosslinkers that connect the random coil and/or helical chains together. The mean field theory model [110] simplifies the structure of silk fibers to order/disorder fractions, which is related to sequence length ratio between β -sheet and random coil formation domains. The Maxwell model [111] simplifies the β -sheet nanocrystals and amorphous structures with elastic springs and viscous dampers. As a result, all models suitable for prediction of the mechanical behavior of silk fibers were also applied to synthetic polymers, such as thermomechanical properties and viscoelasticity.

However, silk fibers feature several unique mechanical behaviors that synthetic polymer materials do not display. For instance, silk fibers have an outstanding ability to resist cracks and defect propagation [112]. Silk fibers often have defects, like cracks, cavities, and tears, with sizes that span from several to hundreds of nanometers [113]. In engineering materials, defects usually are seeds to localize stress (known as stress concentration) and lead to material failure [114], while the stress and strain of failure of the defective silk fibers can converge to defect-free fibers when the crack size is lower than 50% of the width of the fiber diameter. Another unique characteristic of the silk fibers is their toughness at ultralow temperature. Spider silk and wild silkworm silk fibers exhibit ductile failure even at liquid nitrogen temperature (-196°C), and the stress-strain curve does not differ from the behavior seen at room temperature [115, 116]. In contrast, highly stretchable polymer elastomers, such as nitrile rubber, lose elasticity or break immediately once immersed in liquid nitrogen, since the polymer chain segments are frozen when the temperature is lower than their glass transition temperature [115].

Recently, the roles of SNFs in the determination of the mechanical properties of silk fibers have been unraveled to some extent using multiscale computational modeling [7, 112]. The impact of these new insights has included: (i) the nanoconfinement effect in the β -sheet dimension [7] and fibril diameter [112] (Figure 6A–D). The most crucial structure in silk responsible for its exceptional strength is the β -sheet nano-crystals, which cross-link the

semi-amorphous chains of amino-acids and work as reinforcements in the material [117]. The underlying chemical structure of β -sheet is the ordered H-bonds network that bundles the chains of amino acids together. The failure of the β -sheets happens with the deconstruction of the H-bond networks [7, 118–120]. Although the H-bonds are weak interactions, exceptional strength is achieved in silk. Atomistic simulations (Figure 6A–C) show that the failure of the H-bonds happens in a fashion where clusters of 3 to 4 H-bonds break concurrently [120], suggesting the highest strength is achieved in a sequence of multiple short β -sheet strands, but not a single long strand. The effects of nanoconfinement also exist in the cross-section area of the β -sheets (2–4 nm). By applying lateral loading on β -sheet crystals with various thicknesses, atomistic simulations show that the dominant deformation mechanism of the nanocrystal is shear, instead of bending, for smaller crystals [120]. The H-bonds act cooperatively to achieve high strength in this mode and energy is dissipated through the stick-slip mechanism. Besides the β -sheet crystals, semi-amorphous phases also exist in the silk protein. Simulations have been performed to establish the atomistic structure of silk that includes both the crystalline and semi-amorphous phases [121, 122]. The semi-amorphous phase is softer and weaker than the crystalline phase but shows higher extensibility because of the hidden length in the coiled structure [13]. Once the composite of crystalline and amorphous phases are loaded together, the deformation of semi-amorphous phase dominates in the early stage up to the point that it is entirely extended, beyond which the deformation of the crystalline β -sheets will dominate with an apparent strain hardening until eventual failure [13]. At the mesoscale (Figure 6E), the heterogeneous globules/protrusions of fibrils endow several advantageous mechanical behaviors for the regulation of fibril movement, such as deformation and non-slip kinematics, restricted fibril shearing, controlled slippage, stress transfer, as well as energy dissipation (Figure 6E and G) [123–125]. When the silk fibers undergo stretching, the fibrillar structure can inhibit the transverse growth of cracks through longitudinal splitting [125]. The addition of globules in fibrils even allows the tuning of free volume and thus, the wettability of silk (with implications for supercontraction) [124].

This multi-regime deformation process at the atomistic scale is the basis for high strength and toughness of silk, and it is further scaled up to the macro-scale by the nanoconfinement in silk fibrils [112]. By introducing a coarse-grained model of the non-linear response of the silk protein, simulation work has shown that there exists a critical dimension (~ 50 nm) below which the proteins deform synergistically, resulting in enhanced strength, extensibility and toughness at macro-scale [112]. The exceptional mechanical properties also motivate the synthesis in lab, in a way that resembles the delicate spinning process in spiders and silkworms. Atomistic simulations could help to identify the key factors that control the final quality of the synthesized silk fibers. By applying shear flow on the silk protein in ionic solution, the transition dynamics of the secondary structure of protein under the silking process was studied [126]. The shear stress for the transition has been identified to be around 300–700 MPa, which sets a quantitative guideline for the design of devices for synthesizing silks. With a coarse-grained model of the silk protein, simulations can reach the scale that includes massive molecules (a total number of 1,152,000 beads) and help to reveal the dynamics of the formation of the cross-linked network [127]. The results show that an intermediate ratio between the hydrophobic and hydrophilic building blocks is necessary for

building well-connected polymer networks. While shear flow promotes the formation of networks, it also destructs the networks formed by short polymers, suggesting that long polymer chains are also necessary for the formation of robust networks. These key factors have been successfully transformed into synthesizing artificial SNFs [127, 128].

3.4 CoNFs

Collagen is the major component of the connective tissue of vertebrates, serving as a loading system because of its superior mechanical properties [129]. The secret of the outstanding mechanical properties of collagen is its hierarchical structure across multiple scales (Figure 7). As mentioned, the collagen molecule is composed of three polypeptide chains that wind around each other to form a triple helix at the atomistic scale (Figure 7A). The H-bonds formed between the polypeptide chains help stabilize the structure [129, 130], and thus the length of one single tropocollagen molecule can reach ~300 nm. As the tropocollagen is loaded axially, it experiences an entropic extension of the long molecule, uncoiling of the helices, stretching of the covalent bonding in the molecule and eventually failure [130–132]. The most intriguing feature of the structure of collagen material is the staggered assembly of the tropocollagen molecules with regularly distributed gap-regions and overlap-regions, as shown in Figure 7A. In the assembled structure, the interface between tropocollagen molecules emerge and thus the overall mechanical properties of the CoNF will not only rely on the deformation of single tropocollagen molecule but also the interfacial properties. With a full-atomistic model of the full length of tropocollagen (Figure 7B), the atomistic details of the deformation of the assembled CoNFs have been unraveled [133]. For collagen in the hydrated state, the CoNFs initially deform as the straightening of the molecules, which mainly happen in the gap region where molecules are less dense and less organized with more kinks. Further loading will involve the stretching of the molecules both in the gap and overlap region, resulting in higher stiffness. Due to the relaxation of the interface between the tropocollagen molecules, the assembled CoNFs are softer than single tropocollagen molecule, reducing from to 4.8 ± 2 GPa to ~300 MPa [133]. Through atomistic simulations aided theoretical analysis, it was discovered that the strength of the CoNFs is governed by the strength of single tropocollagen or the intermolecular interaction [134]. There exists a critical length scale beyond which the tensile loading on the CoNFs would initiate slip pulses if the intermolecular interaction is a low or brittle fracture of the CoNFs if the intermolecular interaction is sufficiently strong to prevent slip-pulses. Beyond this length scale, the strength and energy dissipation is not increased efficiently, which explained the constant length of ~300nm for tropocollagen molecules found in nature [134]. The results also indicate that the modifications on the intermolecular interactions would result in distinct deformation mechanisms of the CoNFs. In natural CoNFs, cross-links can form between adjacent chains and thus enhance the intermolecular interaction strength [132, 135, 136].

A coarse-grained model of collagen has been developed so that massive simulations (a model consists of 155 tropocollagen molecules which represent a total of 33,790 beads [135]) could be performed on the assembly of many tropocollagen molecules [130, 132, 135]. For less and weaker cross-links, the deformation is dominated by the uniform stretching and then sliding between molecules. As the density and strength of cross-links increase, the load transfer between collagen molecules is enhanced, and the CoNFs deform

synergistically. In this way, the stretching of the collagen backbones would be activated, reaching higher stiffness and strength. Another factor that modifies the interactions between collagen molecules is biomineralization, which is important for carrying loads in bone. A full atomistic model of the mineralized CoNFs (Figure 7B) has been developed by incorporating mineral phases into the full-length model of CoNFs [8, 133, 137]. Atomistic info from the simulations shows that the load transfer between collagen molecules are enhanced by hydrogen bonds and salt bridges formed between collagen and hydroxyapatite. Since most of the mineral phase exist in the gap regions, the stiffness of the gap region would get stiffer as the mineral density increases. As a result, the dominant stretching of the gap region of the non-mineralized collagen will be reduced. Instead, more uniform stretching of both the gap and overlap regions were observed, and the stretching of the overlap region will dominate as the stiffness of the gap region surpasses that of the overlap region. Calculations on the stress and strain distribution within the composite show that the stress is predominately carried by the mineral phase and the deformation mainly carried by the collagen. The mixture of these two materials thus enables enhanced energy dissipation. To extend the study of effects of mineral phase, a coarse-grained model of the mineralized CoNFs has been introduced [130, 132, 135, 138]. Similar to the effects of cross-links, the intermolecular sliding is dominant for less mineral content. For the high content of the mineral phase, the load transfer between collagen molecules is enhanced, and thus the load on single collagen molecule can reach its strength, and the material will fail with the fracture of the collagen molecules.

4. Methodologies of fabrication of biopolymer nanofibrils

In this section, we summarize the strategies for biopolymer nanofibril production with the aim of inspiring new routes of design. These methods can be classified as “top-down” and “bottom-up”. A top-down method is the direct isolation (extraction) of the natural nanobuilding blocks from biological materials. On the other hand, a bottom-up approach is the assembly of biopolymers into nanofibrils. The assembling process can trigger by the changes of pH, solvent, temperature and external forces (e.g., electric and magnetic field).

4.1. Methods of preparation of CNFs

Since the disintegration of cellulose pulps into CNCs and CNFs was developed in the 1950s [139] and 1980s [140, 141], respectively, substantial researches have emerged to enable the production of CNCs and CNFs from higher plants, tunicates, algae and other organisms. Acid hydrolysis and mechanical nanofibrillation are two of the most used methods [18]. However, these methods typically are high energy consumption. For instance, the mechanical nanofibrillation, a process to isolate the nanofibrils by using mechanical devices, usually cost 20,000 ~ 30,000 kW•h/tonne, even reaching up to 70,000 kW•h/tonne in some cases [142]. Accordingly, different chemical- and enzymatic- pretreatments/modifications have developed to decrease energy consumption and improve the nanofibrillation degree in mechanical processing. Currently, the mature protocols for CNC and CNF isolation have been established, and some of the resulting CNC and CNF aqueous solution has been commercialized. Thus, we first introduce these well-established top-down approaches.

4.1.1 Top-down methods—To release CNFs, the matrix such as hemicellulose and lignin should be removed, and the cell walls should be disintegrated via various top-down methods. Mechanical nanofibrillation and chemical modification combined with mechanical nanofibrillation can collapse the cell walls and individualize CNFs with high aspect ratio and web-like entangled structures. In contrast, strong acid hydrolysis introduces stronger attack and hydrolyzes the amorphous regions of cellulose, resulting in short CNCs with a low aspect ratio but with a high specific surface area and high relative crystallinity.

4.1.1.1 Mechanical Nanofibrillation: Mechanical nanofibrillation is mainly dependent on the equipment. High-pressure homogenizers, grinders, blenders, high-intensity ultrasonicators, and twin-screw extruders have been successfully utilized for the exfoliation of CNFs from cellulose pulps. In the early 1980s, the individualization of CNFs from wood cellulose pulps using cyclic mechanical treatment in a high-pressure homogenizer was reported [140, 141], which disintegrated the cellulose pulps into CNFs through a large pressure drop, high shear and impact forces [143–145] (Figure 8A–D). The CNFs exhibited high aspect ratios with web-like entangled structures [145–148]. The length and degree of nanofibrillation of the CNFs highly depended on the applied pressure of the homogenizer, the repeated process times, the chemical pretreatments and the cellulose sources.

The grinder is another prevalent apparatus for producing CNFs [149, 150]. In this technique, the cellulose pulps passed an area between a static grindstone and a rotating grindstone (around 1,500 rpm). During nanofibrillation process, these discs generated strong cyclic shearing forces on pulps and disintegrate them into CNFs (Figure 8E–H) [151, 152]. The structure and degree of nanofibrillation of the CNFs mainly based on the distance between the discs, the repeated processing time, the chemical pretreatments as well as the cellulose sources.

Cellulose pulps can also be nanofibrillated via a high-speed blender [153]. The blender contains a motor and a bottle with a propeller inside. An accompanying tamper can be used to accelerate the blending operation. During high-speed blending, the rotating propeller drives the agitation of the suspension, which generates intense impact on cellulose pulp and results in nanofibrillation into CNFs (Figure 8I and J) [153]. A 0.7 wt% wood cellulose pulp dispersion treated for 30 min in a blender at 37 000 rpm showed high degree of nanofibrillation [153]. During the blending process, the straw-like pulp was nanofibrillated first by forming many “balloon-like” structures. As the balloons extended to the edges, the CNFs were rapidly isolated. However, the pulp fragments with ripped cell walls were split into thinner fragments and gradually disintegrated into CNFs. In this processing, the blending parameters, such as agitation speed, agitation time, pulp concentration and cellulose sources play critical roles in colloid performance, the degree of nanofibrillation of the pulps and the structures of the CNFs.

High-intensity ultrasonication is also intensively utilized for CNF isolation [154–159]. A common ultrasonicator is equipped with a cylindrical titanium alloy probe tip with a tunable diameter (Figure 8K), and usually, the cellulose pulps are suggested to be placed in an ice bath for cooling during the ultrasonication process. The exfoliation of the cellulose pulps was achieved by the acoustic cavitation of high frequency (> 20 kHz) ultrasound in the

formation, growth, and collapse of microbubbles in aqueous solution [160]. The violent destruction induces strong microjets and shock waves on the surfaces of the cellulose pulps, thereby causing erosion of the pulp surfaces and causing them to split along the axial direction for extraction as downsized nanofibrils (Figure 8L) [160]. The exfoliation speed mainly depended on the intensity and frequency of the ultrasonic wave as well as the ultrasonic processing time. Typically, the increase of ultrasonic intensity can increase the efficiency of the nanofibrillation process, and the rise in treatment time helps to achieve a higher degree of nanofibrillation.

For rapid-fabrication of CNFs with high yield, twin-screw extrusion was introduced [161]. The screw of the extruder provides a combination of kneading and feeding, which generates substantial forces to individualize CNFs. Wet cellulose pulps with a high solids content (approximately 25–40 wt%) can be processed through the extruder. The produced CNFs also have a high solids content (up to ~50 wt%). Furthermore, the CNFs obtained from twin-screw extrusion are solid state (powders) instead of an aqueous paste or suspension, which exhibits an advantage for industries when considering transportation, production, and storage issues.

To decrease the energy consumption and to improve the degree of nanofibrillation, mechanical/chemical/enzyme pre-treatment was usually performed. The principal objective of the mechanical pretreatment is to partially fibrillate the cellulose pulps and reduce the particle size to avoid jamming the equipment. Refiner [145], high-intensity ultrasonication [144, 162], and high shear refining [163] has been utilized to reduce the particle size of cellulose pulps. Labscale equipment that composed of a thermostatic reactor coupled to an inline dispersing system also has been designed to divide the pulp fibers into smaller parts [164], which can be nanofibrillated easily through subsequent high-pressure homogenization. Compared with the wood CNF extraction, the exfoliation of cotton celluloses is relatively challenging due to their pure cellulose structure where strong hydrogen bonds exist between the CNFs. Chen et al. [165] used a high-speed blender to pre-treat the cotton cellulose pulps. This process helps to break down the fiber structures and decrease fiber size, allowing the harvest of elongated CNFs with a width of approximately 10–30 nm after high-pressure homogenization.

Chemical pre-treatment was usually utilized to remove lignin and the most of the hemicellulose from cellulose resources, resulting in cellulose pulps with porous structures and relatively weak interactions between the CNFs [152, 166–171]. Therefore, the chemical pretreatments would facilitate the nanofibrillation process and help to prepare the finer CNFs. In 2007, an acidified sodium chlorite solution and a potassium hydroxide (KOH) solution were used to remove lignin and hemicellulose (partially) from wood powder. The subsequent grinder-based nanofibrillation of the as-prepared cellulose pulps produced CNFs with a uniform width of approximately 15 nm [152]. Using a similar technique, CNFs with diameters of 12–35 nm, 12–55 nm, and 15–20 nm, were also successfully extracted using one-time grinding treatment from the chemical pre-treated cellulose pulps of rice straw, potato tuber, and bamboo, respectively [166, 167]. For the dried pulps, the nanofibrillation process became difficult because the drying process generates strong hydrogen bonding between CNFs. Recently, pre-treated, dried cellulose pulps with sodium hydroxide (NaOH)

solutions were reported where NaOH (8 wt%) could loosen the hydrogen bonding between CNFs in the dried pulps [172]. After nanofibrillation by a bead mill, CNFs with a width of approximately 12–20 nm and cellulose I crystal form, were successfully fabricated.

Enzyme pre-treatment is another strategy for facilitating the nanofibrillation process. For example, the treatment of wood cellulose pulps by endoglucanases promoted the disintegration, the CNFs of about 15–30 nm in width and several micrometers in length has been extracted from these pretreated pulps via a high-pressure homogenizer processing [173]. However, a fraction of shorter CNFs was also presented in the samples. Monocomponent endoglucanase was also used to cleave the wood cellulose pulps [174]. Even small additions of the monocomponent endoglucanase enzyme have significantly promoted cell wall delamination and prevented the blocking of the homogenizer. After a refinement (using a refiner) and a high-pressure homogenization, CNFs with a width of ~5–6 nm (single CNFs) and ~10–20 nm (CNF bundles) were produced [174]. Recently, cellulase was used to cut and swell cotton fibers through breakage of the hydrogen bonds between CNFs [175]. After pretreated with cellulase, mainly consisting of cellobiohydrolase, the successful mechanical nanofibrillation of the cotton boll and dried cotton was achieved, resulting in CNFs with a width of 10–50 nm, although a few CNF bundles of around 100 nm remained.

4.1.1.2 Chemical modification combined with mechanical nanofibrillation: Thus far, no mechanical methods (even integrated with pretreatments) can efficiently exfoliate plant cellulose pulps to the single CNF level. Accordingly, a variety of surface chemical modifications of cellulose pulps have been used to facilitate the nanofibrillation [151]. In these routes, the hydroxyl groups of the cellulose were modified and generated new charged chemical groups, which introduced electrostatic repulsion between the CNFs within the cellulose pulps. As a result, the adhesion between CNFs was loosen and can be easily isolated in subsequent mechanical nanofibrillation. A carboxymethylation method was established to modify the cellulose pulps [176]. This treatment increased the anionic charges via the formation of carboxyl groups on the surface of the cellulose and promoted the creation of highly charged cellulose pulps [177]. After further nanofibrillation through a high-pressure fluidizer, individualized CNFs were produced (Figure 9A and B) [178], and presented more uniform dimensions compared to the CNFs obtained without carboxymethylation of the pulps [177].

Since 2006, a novel method to fabricate highly individualized CNFs through a 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)-mediated oxidation modification of cellulose pulps followed by a mild disintegration process was developed [179–182]. TEMPO is water-soluble and stable nitroxyl radical and its chemical structure changes to N-oxoammonium cation and hydroxylamine structures upon oxidation and reduction, respectively. During TEMPO-mediated oxidation, the C₆ primary hydroxyl groups exposed on the CNF surfaces are selectively converted to C₆ carboxylate groups. Owing to electrostatic repulsion and osmotic effects between anionically-charged CNFs, the entirely individualized CNFs can be formed and dispersed in water via gentle mechanical disintegration [183, 184]. For instance, through a combination of the TEMPO-mediated oxidization and gentle mechanical disintegration treatment, wood CNFs were prepared with 3–4 nm in width and a few microns

in length, which corresponded to single elemental fibrils within wood cell walls [180, 183]. The presence of birefringence of the CNF suspension also confirmed the successful preparation of individual CNF dispersions [181]. In addition, using TEMPO-oxidized approaches, the transparent and viscous wood CNFs suspension can be obtained due to their high degree of nanofibrillation and excellent dispersion in water. Other cellulose sources such as cotton linters, bacterial, tunicate, and algal celluloses were also converted to individual CNFs by TEMPO-mediated oxidation, while the optimum oxidation conditions varied between the different original cellulose sources (Figure 9C–F). Typically, the width of the wood CNFs (produced from sulfite wood pulp) and cotton-CNFs (produced from cotton) extracted from TEMPO-oxidized modification are around 3–4 nm (Figure 9C) and 6–8 nm (Figure 9D), respectively. However, Tunicin CNFs exhibited two classes of CNFs (Figure 9E), one with a width of about 10–20 nm and the other one with a lateral size comparable to that of cotton CNFs (6–8 nm) [181].

Compared to mechanical nanofibrillation, a combination of TEMPO-mediated oxidation modification and mechanical treatments present advantages, because the TEMPO-mediated oxidation can introduce negative charges on the CNF surface. The followed mechanical nanofibrillation becomes relatively easy and requires much lower energy consumption. The processing can even be carried out through agitation using a magnetic stir bar at 1500 rpm in an airtight bottle [180]. Unfortunately, several physical properties of CNFs were weakened by the TEMPO-oxidized modification. For example, the thermal stability of the wood TEMPO-oxidized CNFs was much lower than that of CNFs produced from a grinder or a blender [165, 185, 186]. Further, the toxicity of the TEMPO-oxidized system is another issue required for further evaluation.

4.1.1.3 Strong acid hydrolysis: Strong acid hydrolysis is an effective method to produce CNCs from various cellulose sources. During strong acid hydrolysis, the degree of polymerization of the cellulose was rapidly decreased. The disordered or paracrystalline regions were preferentially hydrolyzed, whereas crystalline parts that had a higher resistance to acid attack remained intact. Therefore, after a strong acid treatment that hydrolyzes the “defect” parts of the cellulose, rod-like CNCs were produced. Due to the hydrolysis capability by strong acids, CNCs can be fabricated from almost all cellulosic materials. Most common cellulose sources for strong acid hydrolysis include cotton, wood cellulose pulps, ramie, flax, hemp, sisal, crops straw, microcrystalline cellulose, bacterial cellulose, and tunicate. In the 1950s, it was first reported that colloidal suspensions of CNCs could be produced by controlled sulfuric acid (H_2SO_4)-catalyzed degradation of cellulose pulps [187]. These CNCs could form birefringent gels and liquid crystalline structures due to their rod-like features [139]. Detailed liquid crystalline characteristics were reported and revealed that the CNCs appeared with chiral nematic ordering in aqueous suspension and exhibit helicoidal self-ordering behavior [188, 189].

In a typical acid hydrolysis process, the cellulosic materials were first immersed in strong acid for hydrolysis under controlled experimental conditions (such as specific temperature, processing and time). Next, the various operations were conducted on diluted suspensions to remove the acid. These processes include washing the suspension repeatedly by centrifugation, filtration, dialysis against distilled water, as well as the integration of the

steps mentioned above. To fabricate highly individualized CNCs, the as-prepared samples were further treated with mechanical disintegration devices, such as magnetic stirrers, high-pressure homogenizers, high-speed blenders and high-intensity ultrasonicators. The structure of the as-prepared CNCs was mainly related to the crystal sizes along the longitudinal direction of the cellulose chains present in the original cellulose sources. However, experimental conditions, such as the types and concentrations of the acids, the acid-to-cellulosic material ratio, temperature, agitation time, and the mechanical treatments, also had impacts on the structures of the resultant CNCs.

Sulfuric acid is the most widely used reagent for CNC preparation. During the hydrolysis process, this acid reacted with the surface hydroxyl groups of cellulose to yield charged surface sulfate esters which promoted dispersion of the CNCs in aqueous solution. However, the introduction of charged sulfate groups led to an apparent decrease in thermal stability of the CNCs. The concentration of H_2SO_4 for hydrolysis was usually set at approximately 65 wt%, while the temperature was tunable (from room temperature to 70°C), and the treatment time also varied from 30 min to overnight. Typically, hydrolysis at high temperature with an extended processing time is suitable for producing short and highly-crystallized CNCs, but the experimental process should be carefully conducted and monitored to avoid the carbonization of cellulose by dehydration. Also, the surface charge of CNCs is highly sensitive to heat, as an increase in temperature can cause the de-esterification of the sulfate groups. The particle size, surface charge and the polydispersity of the CNCs changed gradually with the rise in the degree of acid hydrolysis [190]. A frequently-used H_2SO_4 hydrolysis process has been established where the filter paper powder (cotton cellulose) was chosen as a cellulosic source, and 64% (w/v) H_2SO_4 was added into powder with pulp to acid ratio of 1:8.75 [190]. The resultant cellulose pulps were incubated at 45°C for 1 h, followed by 5 min ultrasonic treatment. CNC suspensions prepared in this manner readily formed an anisotropic phase above a critical concentration of CNC (around 4.5% w/v). Longer hydrolysis times led to shorter rod-like CNCs with narrower particle length distributions, while a higher acid-to-pulp ratio decreased CNC dimensions to some extent at the reaction time of 45 min [191]. Furthermore, the structures of the CNCs were strongly related to the cellulose sources used for processing. The shape and size distribution of CNC prepared from cotton, Avicel, and Tunicate was presented in Figure 10A–C [192]. The cotton CNCs had a length ranging from 25 to 320 nm and a width from 6 to 70 nm, while the length decreased significantly when the hydrolysis temperature was increased (Figure 10A). The length of Avicel CNCs varied from 35 to 265 nm while the width changed in the range of 3 to 48 nm (Figure 10B). Tunicin CNCs were several micrometers long and had a whisker-like morphology with kinked defects, which were the localized damage of the CNCs generated in the sonication treatment (Figure 10C) [192]. The kinks in long tunicin CNCs usually result from the mismatch of the amorphous and crystalline regions along the CNC direction.

Hydrochloric acid is another strong acid has been intensively used for CNC isolation. Different from H_2SO_4 , HCl does not react with the cellulose, so the final CNCs remain with dominating hydroxyl groups on their surfaces. The CNCs produced from HCl hydrolysis appeared short but without highly individualized morphologies, and a significant amount of CNC aggregates can be observed in suspensions. To overcome this issue, a modified route

was reported to extract CNCs from bleached softwood kraft pulp by using a 4N HCl [193]. The CNCs yielded from this hydrolysis protocol appeared virtually free of surface charges and could be isolated at single CNC levels approximately 3.5 nm wide and 180 ± 75 nm long. The production of CNCs without ionic surface groups was also reported by hydrolysis of tunicate cellulose pulp through a 3N HCl treatment and subsequent lyophilization [194]. The lyophilized CNCs with width of approximately 19.9 nm could re-disperse in acidic protic solvents, like formic acid and m-cresol, due to their strong capability to break the hydrogen bonds between CNCs (Figure 10D). As no chemical modification was introduced during the hydrolysis process, the HCl-hydrolyzed CNCs showed a much higher thermal stability than that of H_2SO_4 -hydrolyzed CNCs (Figure 10E). For example, the HCl-hydrolyzed CNCs exhibited high thermal degradation temperatures of 341.7°C , whereas the thermal degradation of H_2SO_4 -hydrolyzed CNCs started at $\sim 120^\circ\text{C}$ and occurred over a broader temperature range [160].

Phosphoric acid hydrolysis can also be used to prepare thermal-stable CNCs. The fabrication of slightly phosphorylated CNFs (surface charge density was 10.8 ± 2.7 mmol $\text{PO}_4^{-2}/\text{kg}$ cellulose) through the controlled hydrolysis of cotton with H_3PO_4 was reported [195]. The as-prepared CNCs displayed an average width of 31 ± 14 nm, an average length of 316 ± 127 nm, and an average aspect ratio of 11 ± 1.5 (Figure 10F). The CNCs were readily dispersible and formed stable dispersions in polar solvents such as water, dimethyl sulfoxide, and N, N-dimethylformamide. The H_3PO_4 -hydrolyzed CNCs exhibited high thermal stability with the weight loss mark at 290°C and the maximum thermal decomposition temperature at 325°C [195].

4.1.2 Bottom-up methods—Several bottom-up methods were developed to fabricate CNFs through dissolution of cellulose followed by regeneration and processing. In these methods, cellulose was first dissolved in solvents such as N-methylmorpholine-N-oxide, ionic liquids, LiCl/N, N-dimethylacetamide, NaOH aqueous solution, alkali/urea and NaOH/thiourea aqueous solution [196]. Then, various techniques, e.g., supercritical drying [197] and electrospinning [198], were used to regenerate CNFs from cellulose solution. Another bottom-up approach to fabricate CNFs is the synthesis of BCs by bacteria [24, 143, 199–204]. Instead of being obtained by nanofibrillation of large-sized cellulose pulps, BC is reversely produced by bacteria, synthesizing cellulose and building up of CNFs (Figure 11A). In contrast to the top-down methods, most of which generate CNFs with a low yield, the production of BC is more convenient to scale-up, for instance, many food companies can produce several tons per day [24]. BC has long been utilized as the raw material of *nata-de-coco*, where a one-centimeter thick gel sheet can be generated by fermenting the bacteria with coconut water and immersed in sugar syrup [199]. In recent years, to find more environmentally friendly approaches and to reduce the production costs for BC, attention has transferred to utilize agricultural and industrial wastes as alternative nutrient sources [205]. Compared with CNFs produced from cellulose pulps, BC is more chemically pure, contains no hemicelluloses or lignin, so extra processing to remove these unwanted impurities is not required. BCs have a high aspect ratio, with a diameter of 20–100 nm and have a tight, mesh-like structure that provides high porosity and a large specific surface area, thus enhancing their applicability as engineering materials [206]. BCs also feature other

advantages, such as high crystallinity (70–80%), a high degree of polymerization (up to 8,000 [207]), high water content (to 99% [205]), moldability, excellent biocompatibility, hydrophilicity, and nontoxicity.

Several bacteria synthesise cellulose[208], including strains from the genera *Acetobacter*, *Agrobacterium*, *Pseudomonas*, *Rhizobium* and *Sarcina*. The most efficient producer of BC is *Acetobacter xylinum*, as was first reported in 1886 [209]. This nonphotosynthetic organism can utilize glucose, glycerol and other organic substrates and convert them into pure cellulose [210]. The biosynthesis of BC in *A. xylinum* occurs between the outer membrane and the cytoplasmic membrane by a cellulose-synthesizing complex, which is in association with pores at the surface of the bacterium. The process includes the synthesis of uridine diphosphate-glucose, which is the cellulose precursor, followed by the addition of this intermediate to the end of the growing cellulose molecule. The synthesized cellulose chains exit the cell as a so-called elementary fibril through pores at the bacterium surface (Figure 11C–E) [211]. The BCs excreted by the bacteria self-assemble to ribbon-like structures and eventually 3D nanofibrillar networks (Figure 11F and G) [212]. The cellulose synthase is considered to be the most important enzyme in this process [199, 210]. It is estimated that 1 bacterium can convert 108 glucose molecules per hour into cellulose [205].

A classic protocol to grow BC in laboratories was developed [199]. First, a culture medium was prepared by dissolving 50g sucrose, 5g yeast-extract, 5g (NH₄)₂SO₄, 3g KH₂PO₄, and 0.05g MgSO₄·7H₂O in a liter of water. Second, an aliquot of activated seed broth was added into the culture medium and further cultured in a mixture in a static condition at around 28~30°C. During the incubation, the thickness of the BC layer (a white pellicle) increased steadily with time, and can reach up to 25 mm for 4 weeks incubation (Figure 11B). BC can also be fabricated in agitated culture, and it accumulates in dispersed suspension as irregular masses, such as a granule, stellate, and fibrous strands [210, 213]. Many process parameters that disturb the assembly of primary nanofibrils at the bacterial surface can affect the structure and physical properties of the BCs. These parameters include pH, temperature, incubation time, carbon and nitrogen sources, oxygen and carbon dioxide pressures, additives, agitation and drying [199, 205]. To obtain pure BC, the as-prepared nascent BC gels can be wash within dilute alkaline solution and water to remove bacteria entrapped in the gels [199].

4.2 Methods of preparation of ChNFs

The interest in chitin as a material began in the 1920s when market pressure for low-cost fibers promoted research on artificial silks, but they were being replaced by synthetic polymer fibers [214]. Since the 1970s, chitosan, the closest chemical entity of chitin, dominated related research due to its excellent water solubility, biocompatibility, process accessibility, and positively charged features. Chitin, however, is water-insoluble and thus more difficult to process. Chitin was considered a food and industrial waste. In recent decades, the preparation of ChNFs has increased primarily due to the biological and physicochemical advantages [215]. The techniques that used for CNF isolation are often used here, and the routes to prepare ChNFs also can be classified as top-down and bottom-up route [216–219]. In this section, the most recent progress on ChNF preparation,

especially methods developed in the last decade, are reviewed with an emphasis on top-down approaches.

4.2.1 Top-down methods—Owing to the chemical- and structural- similarities between CNFs and ChNFs, many practices employed for extraction of CNFs have been transferred to prepare ChNFs in the last decade [220–225]. These methods also can be divided into the following three main groups: (1) mechanical nanofibrillation; (2) chemical modification combined with mechanical nanofibrillation; (3) strong acid hydrolysis.

4.2.1.1 Mechanical Nanofibrillation: The ChNFs prepared by mechanical nanofibrillation are usually characterized by long length and high aspect ratio, while the widths can be diverse due to the different chitin sources and mechanical treatment processes used. Recently developed representative mechanical nanofibrillation methods are shown in Figure 12. Ultrasonication is an early mechanical nanofibrillation method. In 2007, an ultrasonic procedure was used to extract ChNFs with widths of 25–120 nm by treating chitin in water at neutral conditions with ultrasonication (20 kHz, 900–1000 W) for at least 30 min [159]. Similarly, α -ChNFs was fabricated from shrimp shells with widths of 20–200 nm via a simple high-intensity ultrasonic treatment (60 kHz, 300 W, pH=7) for 30 min, and transparent films and soft foams were prepared from the ChNFs [226]. The ultrasonic technique was also applied in acidic conditions [227]. Individualized ChNFs with widths of 3–4 nm and at least a few microns in length were prepared by ultrasonication (19.5 kHz, 300 W) of squid pen β -chitin in water at pH 3–4 for a few minutes. In this procedure, a shorter ultrasonic time and lower energy were required, and ChNFs with thinner widths were obtained compared to the previous case. The protonation (cationization) of the C₂ amino groups present on the crystallite surfaces of the squid pen β -chitin (due to the acidic conditions) likely provided electrostatic repulsive forces between ChNFs, an essential state for nanofibril conversion. Other conditions may include the relatively low crystallinity, parallel chain packing mode, and relatively weak intermolecular forces of squid pen β -chitin, which are different from those of α -chitins.

Grinding is another commonly-used mechanical nanofibrillation method. ChNFs were prepared with widths of 10–20 nm and high aspect ratios by grinding wet or dried chitins originating from crab shells at 1500 rpm at pH 3, and transparent films of ChNFs/acrylic resin composites were prepared from these materials [228]. As the case of disintegrating squid pen β -chitin at pH 3–4, the mechanical treatment in acidic conditions is the key to successfully fibrillating chitin. The cationization of amino groups on the chitin surface broke the strong hydrogen bonds between the ChNFs due to electrostatic repulsion. By applying the same grinding procedure, they also isolated ChNFs from mushrooms with widths of 20–28 nm [229].

A starburst system (1–15 passes) has also been used to isolate crab shell α -ChNFs with widths of approximately 20 nm at either neutral or acidic conditions (with or without adding acetic acid) [230]. The morphology of the ChNFs depended strongly on the number of passes through the apparatus, and acidic conditions improved the chitin fibrillation, resulting in thinner ChNFs with fewer pass times. Such findings again demonstrated that the addition of acetic acid could significantly enhance the efficiency of nanofibrillation of chitin.

Meanwhile, when chitin purified from prawn shells was subjected to grinding treatment at 1500 rpm, ChNFs of approximately 10–20 nm wide were successfully extracted without adding acetic acid [231]. The exoskeleton of the prawn shell consists of a finer structure than that of crab shell, which allows for ChNFs to be prepared under neutral pH conditions.

Therefore, the following three factors were considered to dominate the efficiency and outcomes of mechanical nanofibrillation processes: (i) the chitin source, (ii) the adopted apparatus, and (iii) the pH conditions. Generally, (i) lower crystallinity, weaker hydrogen bonds and finer exocuticle structures allowed for easier disintegration of ChNFs (such as in chitin from squid pen and prawn shell); (ii) higher intensity and energy consumption result in a higher degree of nanofibrillation; (iii) acidic pH conditions facilitated the nanofibrillation process. Representative morphologies of ChNFs prepared by mechanical nanofibrillation are shown in Figure 13.

Other instruments, such as high-speed blenders, high-pressure homogenizers, microfluidizers or their combinations have also been adopted to isolate ChNFs. For example, dynamic high-pressure homogenization (1000 bar, 40 passes) has been applied to separate ChNFs from lobster and produced the ChNFs with a uniform width (< 100 nm) and high aspect ratio [232]. By using a joint mechanical nanofibrillation procedure which combined grinding (10 passes), microfluidization (30,000 psi, 120 mL/min, 10 cycles) and homogenization, ChNFs were prepared with widths of approximately 50 nm [233].

Most ChNFs are prepared from purified chitin by the complete removal of protein and mineral salts from crustacean shells. Recently, protein-containing ChNFs were also prepared [234]. Here, the procedure included microfluidization (5 passes at 900 and 1600 bar successively) to isolate low protein content-individualized chitins from lobster exoskeletons with widths of 3–4 nm. Strong correlations were observed between low residual protein content and high tensile properties of the resulting protein-ChNF membranes. A protein-ChNF complex was prepared from mineral-removed crab shells by grinding (1500 rpm) and high-pressure water jet atomization (200 MPa, 30 cycles) [235]. The protein-ChNFs were also available as reinforcement fillers to increase the mechanical properties of acrylic resins, and the protein on the ChNF surfaces accelerated the biomineralization of calcium carbonate crystals. Same as CNF nanofibrillation, one advantage of mechanical nanofibrillation process is that it can yield ChNFs with high aspect ratios without the need for any chemical reaction. However, disadvantages include high energy consumption and sometimes incomplete individualization of the ChNFs.

4.2.1.2 Chemical modification combined with mechanical nanofibrillation: To improve the efficiency of ChNF isolation, the chemical modification was performed on the chitin before mechanical treatment. TEMPO-mediated oxidation and partial deacetylation are the two primary chemical modification methods for introducing functional groups on the surfaces of ChNFs to provide electrostatic repulsion, further promoting nanofibrillation during the subsequent mechanical treatment (Figure 14). In the case of TEMPO-mediated oxidation, the selective formation of C₆ carboxylate groups from the C₆ primary hydroxyl groups on chitin crystallite surfaces is the crucial step for preparing individualized chitin nanocrystals (ChNCs, also termed as chitin nanowhiskers) [180]. When TEMPO-mediated

oxidation forms a sufficient amount of carboxylate groups, the anionically charged surface enhances the individualization of the crystallites by ultrasonic treatment through electrical repulsion between the crystallites and/or through osmotic effects in a similar manner to that of CNFs prepared by TEMPO-mediated oxidation (Figure 14). In 2008, [236] a NaClO/NaBr/TEMPO system was used to oxidize α -chitin originating from crab shells at pH = 10, and ChNCs were dispersed in water by the continuous ultrasonic treatment after only a few minutes. The carboxylate content of the TEMPO-oxidized chitins correlated with the amount of NaClO added and further affected the resultant weight ratio of water-insoluble fractions, including shape, length, and width of the obtained chitin nanocrystals. When 5.0 mmol of NaClO (per gram of chitin) was used, the weight percentages of the water-insoluble fractions were as high as 90%, and the carboxylate content reached 0.48 mmol/g. The mostly individualized ChNCs were obtained with average lengths and width of ~340 and ~8 nm, respectively. This TEMPO-mediated oxidation system was also applied to oxidize high-crystallinity β -chitin from tubeworms. The oxidized tubeworm β -chitin featured carboxylate groups at a concentration of 0.18–0.25 mmol/g with yields of > 70% when 2.5–10 mmol NaClO per gram of chitin was added [237]. The oxidized tubeworm β -chitin was further subjected to ultrasonication for several minutes, and nanofibrils 20–50 nm in width and micron-scale lengths were obtained [237]. Apart from TEMPO-mediated oxidation, the esterification of chitin using maleic anhydride and utilizing the C₆ primary hydroxyl groups in chitin was reported [238]. Accompanied with grinding treatment, negatively-charged ChNFs 45 ± 8 nm in diameter were prepared in aqueous solution.

In the case of partial deacetylation, the acetyl groups of acetamide at the C₂ position on chitin crystallites surfaces were partially removed to expose more C₂ amino groups, further providing cationically charged surfaces in acidic conditions [180]. Therefore, based on the same principle of nanofibrillation of TEMPO-oxidized chitin, the cationic groups on the surface enhanced the individualization of the crystallites through electrical repulsion after mechanical treatment under acidic conditions (Figure 14). In 2010, crab shell α -chitin was treated with 33% NaOH at 90°C for 2–4 h, the DNAC (degree of N-acetylation) was reduced from 0.90 to 0.70–0.74 [239]. This DNAC value in partially-deacetylated chitin corresponds to a C₂-NH₂ content of 1.34–1.56 mmol/g, the yield of partially deacetylated chitin was 85–90%, and the crystallinity and crystal size of the original α -chitin were mostly maintained after 33% NaOH treatment. These results indicated that partial deacetylation took place selectively on the α -chitin crystallite surface. When the partially deacetylated chitin was subjected to ultrasonic treatment at pH = 3–4 (adjusted using acetic acid), the nanofibrillated chitin had average widths and lengths of 6.2 ± 1.1 and 250 ± 140 nm, respectively. In addition, individual α -ChNFs of >500 nm in length were observed [239]. Therefore, this prepared nanofibrillated chitin was a mixture of ChNCs and ChNFs. The thinner ChNFs were obtained by lower energy consumption, e.g., higher nanofibrillation efficiency, when compared to the crab shell α -chitin prepared by sole mechanical treatment without deacetylation as described earlier. The protonation (cationization) of the C₂-NH₂ groups in the partially deacetylated chitins to provide cationic charges in high density on the crystalline nanofibril surfaces, (associated with partial mechanical scission of the nanofibrils during disintegration) was the critical driving force for the individualization of α -ChNFs. Partially deacetylated chitin was dispersed in water at acidic conditions by adding various

organic and inorganic acids, and the effects of pH and ionic strength on the nanofibrillation efficiency of partially deacetylated chitin were described [240].

Representative images of ChNFs prepared by chemical modification combined with mechanical treatment are shown in Figure 15. The chemical modification improved the nanofibrillation efficiency and endowed additional functionality to the ChNFs. For example, TEMPO-oxidized and partially deacetylated ChNFs pose the anionically- or cationically-charged surfaces, respectively. Therefore, the hydrogels made by TEMPO-oxidized and deacetylated ChNFs showed different pH-dependent swelling behavior and adsorption affinity due to their various surface charges [241]. And the deacetylated ChNF films showed specific inhibitive effects on the growth of *Escherichia coli* [242]. The anionically- and cationically-charged nanofibrils can be integrated into a material system through layer-by-layer deposition, for instance, TEMPO-oxidized CNFs and partially deacetylated ChNFs has been assembled into multifunctional films with wetting and adsorption properties as well as anti-reflection properties [243]. More recently, the preparation of zwitterionically-charged ChNFs by a combination of both TEMPO-mediated oxidation and partially deacetylation, followed by mechanical treatment, was reported. These ChNFs decorated with both cationic (NH_3^+) and anionic (COO^-) groups on the surface, and can be served as an environmentally and doubly pH-responsive Pickering emulsifier to replace conventional pH-responsive Pickering emulsifiers, e.g., inorganic nanoparticles or grafted polymers [244, 245].

The enhanced properties of nanocomposite films, beads and biological adhesives obtained by using composites of partially deacetylated ChNFs with chitosan or chitin derivatives have been reported [246–248]. Amidated chitin synthesized by the reaction of the amino groups in a partially deacetylated chitin with N, N-dimethylacetamide dimethyl acetal, which was subjected to gas bubbling and ultrasonic treatment, showed nanofibrillation-agglomeration-renanofibrillation behavior during processing [249]. One principal disadvantage of the chemical modification is the processes often requires chemicals that are not environmentally friendly. Enzyme modification, such as by oxidase, deacetylase or specific hydrolase treatment, may be an attractive option to address this issue. Recently, a TEMPO/laccase/ O_2 system has been used to prepare CNFs, and such system was confirmed that applicable to the ChNF isolation [250].

4.2.1.3 Strong acid hydrolysis: Same as the strong acid hydrolysis of cellulose, during acid hydrolysis of chitin, the amorphous domains are removed, the fibrillar structure is loosened, and nanocrystals are produced by subsequent mechanical treatment. Therefore, the ChNFs are characterized by their shorter length, high crystallinity, and rod-like structure, denoting themselves as ChNCs [218]. Typically, acid hydrolysis of chitin was conducted using HCl (e.g., 3 M HCl) at its boiling point (90–105°C) under vigorous stirring for 1.5–6 h. The suspension was diluted with deionized water to quench the reaction and then underwent a series of separations (centrifugation and/or filtration) and washing steps. To ensure that no residual acid was persisted, the suspension was transferred to dialysis membranes and dialyzed against deionized water for several days. Most of the ChNCs prepared from acid hydrolysis had widths of 10–50 nm and lengths of 150–600 nm. However, the length of ChNCs adapted from riftia tubes reached 2,200 nm [218]. ChNCs also were isolated from shrimp shells by acid hydrolysis, and their surface areas have been

measured dye adsorption [221]. The specific area of these ChNCs was approximately 350 m²/g. Representative transmission electron microscopy (TEM) images of ChNCs prepared by acid hydrolysis are shown in Figure 16. The nematic liquid crystal order was observed for ChNCs prepared by acid hydrolysis of purified chitin in boiling 2.5 M HCl followed by strong mechanical treatment. Accordingly, acid hydrolysis is also a conventional process for producing nematic liquid crystal ChNCs [139].

Owing to the loss of amorphous material and the depolymerization of chitin during acid hydrolysis, a well-known disadvantage of strong acid hydrolysis method is the low yield (as low as 60%) [224]. Both the starting material and preparation method can also influence the nanofibrillation efficiency as well as the characteristics of the products, including morphology, crystalline form, and crystallinity, which further affects the material physical properties. In comparison, the films prepared from squid pen β -ChNF dispersion showed the highest shear stress and viscosity due to their highest aspect ratio, while the films of partially deacetylated α -ChNFs had the highest tensile strength because of the antiparallel structure of α -chitin and the higher aspect ratio compared to TEMPO-oxidized and acid hydrolyzed α -ChNFs [251] [251]. The role of acid hydrolyzed ChNCs, and mechanically-treated ChNFs on the mechanical properties of thermoplastic starch films was studied, and the nanocomposite films prepared with ChNFs showed higher Young's modulus and tensile strength due to the web-like morphology [252]. Studies of the strength of single ChNFs via sonication-induced fragmentation found that both squid pen β -chitin and tubeworm β -chitin had similar strengths (~ 3 GPa), higher than that of algal α -chitin (1.6 GPa) [253]. In this case, the molecular packing modes (crystalline form) of chitin governed the tensile strength of the ChNFs, rather than the cross-sectional dimensions and crystallinity.

4.2.2 Bottom-up methods—A variety of “bottom-up” techniques, such as drawing, template synthesis, phase separation, sea-island composite spinning, and modified melt-blowing processes, have been used to assemble chitin molecules into ChNFs [254–259]. Electrospinning can also be considered a bottom-up approach to produce nanoscale chitin assemblies, but these materials should be defined as nanofibers (instead of nanofibrils) more precisely, because these nanofibers are usually 100 times larger than the width of natural ChNFs. Also, the electrospun materials usually are matts instead of aqueous dispersion, thus difficult for further processing [216]. Therefore, we only focus on introducing the solvent-induced self-assembly, which can obtain the ChNFs with width and length that comparable with the natural ChNFs.

Although chitin is difficult to dissolve in common solvents, several solutions such as, hexafluoroisopropanol (HFIP), 1-ethyl-3-methylimidazolium acetate (a kind of ionic liquid), alkali/urea solvent systems, and methanesulfonic acid has been confirmed that having ability to dissolve chitin [219, 260–263]. Particularly, chitin molecules in HFIP solution are able to assemble into 3 nm ChNFs upon drying [264], thus the chitin/HFIP solution system can be directly used as “ChNF ink” to print the ChNF on different substrates with arbitrary shapes. Recently, ChNFs and nanospheres were prepared by dissolution of chitin in H₃PO₄ and regeneration in water [265]. The prepared ChNFs were 20 nm in width. To produce well-organized structures, self-assembly of ChNFs is particularly attractive when complemented by gentle top-down fabrication methods, but on the other hand, bottom-up approaches

include the dissolution or melting of chitin, which make it difficult to prepare ChNFs with high crystallinity, a key disadvantage compared to the top-down approaches.

4.3 Methods of preparation of SNFs

Compared to the advances with CNFs and ChNFs, SNFs, especially those produced from *B. mori* silk fibers, as a nanomaterial building block for particle applications is behind. However, SNFs have received scientific attention in recent years due to their availability, sustainability, biocompatibility, and biodegradability. A variety of bottom-up [266–272] and top-down approaches [159, 273–275] have been developed to isolate and regenerate SNFs from silk fibers (Figure 17). In comparison with conventional silk materials produced from silk fibroin solution [21], natural and regenerated SNFs maintain the sophisticated meso-architectures found in natural silk fibers, and endow regenerated silk materials with new structures and properties that originate from nanosize effects, such as an extracellular matrix-like texture [276–279], nanoporous structure [267, 280, 281], nanoconfinement effects [112, 114, 282] and optical transparency [267, 280]. In this section, we first outline the recently emerged top-down techniques for silk nanofibrillation and further highlight the self-assembly of silk fibroin for obtaining SNFs.

4.3.1 Top-down approaches—As mentioned in section 4.4.1 and 4.4.2, CNFs and ChNFs have been utilized more than half a century [139–141]. However, direct isolation of SNFs from natural silk fibers remains in its infancy. For the conventional silk fibroin material processing, lithium bromide (LiBr)/H₂O [21], N-Methylmorpholine N-oxide (NMMO)/H₂O [283–287], CaCl₂/EtOH/H₂O [288–291], and HFIP [292] dissolution methods usually serve as a starting point, which dissolves silk fibers into silk fibroin molecules, even with partial degradation. However, the structural hierarchy of natural silk fibers, an important element in determining bulk material properties, is destroyed during these dissolution processes. Thus, the physical properties of resulting materials are much weaker than those found in the natural silk fibers. Several physical and chemical methods have been reported for the successful isolation of silk fibers at the nano- or micro- scales. The initial attempt was by directly using high-intensity ultrasonic techniques to exfoliate (break) spider dragline silk and *B. mori* silks [159]. These natural silk fibers were exfoliated into nanofibrils with diameters in the range of 25–120 nm (Figure 18A) after intensive treatment; 1000 W for 45 min for spider silks and 900 W and 30 min for silkworm silks. The resultant SNFs appeared as mats or aggregates (Figure 18A), which could not be dispersed in solution, thus limiting re-processing into useful materials. Formic acid/CaCl₂ dissolution is another practical method to dissolve silk fibers to SNFs [275]. The diameters of these SNFs were in the range of 20 to 200 nm (Figure 18B), and the nanofibrils could be processed into films and electrospun nanofibers. Most recently, a liquid exfoliation strategy to extract SNFs from the degummed silk fibers was designed [273]. This method integrated chemical (partial dissolution by HFIP) and physical (ultrasonic dispersion) treatments (Figure 17A). After this synergetic processing, the silk fibers were isolated to single nanofibrils with diameters of 20 ± 5 nm and contour lengths up to 300–500 nm (Figure 18C). These nanofibrils present outstanding dispersion features and stability in water and can further processed into functional materials. Most recently, a solvent system to produce SNF solutions with tunable features was reported (Figure 18B) [274]. In this process, a

combined solvent system consisting of LiBr and formic acid was tuned to control the degree of the disintegration of hydrogen bonds among the silk fibroin chains and to extract SNFs from silk fibers (Figure 18D). Interestingly, the SNFs generated by this method were mainly composed of amorphous structures instead of the β -sheet rich structure observed in other SNFs.

4.3.2 Bottom-up methods—Compared with synthetic polymers or other biopolymers (including cellulose and chitosan), a unique feature of silk fibroin is the inherent tendency to assemble into sophisticated structures due to the water-solubility and highly-ordered primary structure (consisting of highly-repetitive hydrophobic-hydrophilic domains) [43, 293]. The self-assembly processes of the silk fibroin can be triggered by external stimuli. Conditions that induce conformational transitions of silk fibroin include electric fields, pH, ions, mechanical shear, ultrasound treatment, heat, and ethanol (EtOH) [266–272]. Here, we also excluded the silk nanofibers produced from electrospinning, the related topics have been reviewed [294]. Regarding SNF self-assembly, heat-induced [266, 281, 295] and EtOH-induced methods [267, 296] have been extensively studied (Figure 17C). However, early studies about the self-assembly of silk fibroin mainly focused on understanding the natural spinning mechanisms or the structure-property relationships of silk fibers [268, 269]. Only limited studies attempted to use SNF hydrogels for cell scaffolds, while the mechanical performance of these hydrogels was limited due to the negligible fibril-fibril interactions [297]. More importantly, these hydrogels brittle and hard to process into other useful formats, such as films and fibers. Recently, silk fibroin aqueous solutions with a protein concentration lower than 0.3 wt% could be assembled into elongated nanofibrils with beaded-like topological structures (a diameter of 3–4 nm and the contour length up to 1 μ m, Figure 18E and F) with morphologies comparable to amyloid fibrils (another well-studied β -sheet nanofibril) when incubated in 7 vol% aqueous EtOH solution for 1–2 days or directly incubated at 60°C for 7 days (Figure 17C) [267]. The resultant SNFs could be dispersed homogeneously in water and stabilized as nematic textures. Thus, these preparations could be blended with other materials in aqueous solution. For example, this SNF aqueous dispersion can be mixed with amyloid fibril solutions at desired weight ratios. Due to the simultaneous presence of two mechanically complementary nanofibrils (SNFs and amyloid fibrils, featured by high toughness and modulus, respectively), the molecular, mesoscopic and continuum properties of the final protein nanocomposites were tunable by controlling the weight ratios of these two protein nanofibrils. In addition, SNF hydrogels prepared from silk fibroin concentrations of 0.5–2 wt% were “flowable hydrogels” (named as a reversible hydrogel-solution system). The hydrogels could be reversed back to the solution state at lower concentrations and further gelled after concentration [272].

Inorganic/synthetic nanomaterials, such as graphene oxide (GO) [298], reduced-GO [299, 300], graphene [301], molybdenum disulfide [302] and silicon dioxide [298] have also been confirmed to induce and regulate the self-assembly of silk fibroin. For instance, silk fibroin can be directly grown on the surface of single reduced GO nanosheets (Figure 18G) [299]. The assembled SNFs covered the reduced GO surface completely, offering a soft and hydrophilic surface on GO nanosheets, making the reduced GO stable in aqueous solution and providing further options for functionalization of GO through chemical modification of

the SNFs [299, 300, 303]. Similar self-assembly processing was also detected with other proteins (peptides)/graphene (graphite) systems[304]. AFM characterization revealed that the graphene (or graphite) surfaces adsorbed monomers and oligomers of proteins and further promoted the formation of β -sheets and template fibrillation [304]. These protein/graphene hybrids combined the physical properties of both constituents and could be formed into different functional nanocomposites through simple fabrication processes, such as vacuum-filtration [299], heating- or non-solvent induced gelation [305], and freeze-drying [21].

Integration of experimental and theoretical methods has been applied to address silk fibroin adsorption on hydrophobic-hydrophilic surfaces of GO with different degrees of oxidation [298]. These findings revealed that protofibrils formed at low concentrations, while variance in the deposition speed had a limited effect on secondary structure and morphology of the silk fibroin. Further, the balance of non-bonded interactions between van der Waals and electrostatic contributions help with the retention of silk secondary structure on GO surfaces. Molecular dynamics simulations of silk fibroin on GO, reduced GO, and silicon dioxide surfaces confirmed that strong van der Waals interactions between silk fibroin and inorganic surfaces play a critical role in disrupting secondary structure on these surfaces (Figure 18H). However, compared with GO and silicon dioxide surfaces, the graphene surface may have distinct interactions with silk fibroin. As shown in Figure 18H, the average height of silk fibroin on both GO and silicon dioxide surfaces was similar to that of β -sheet crystal structures. The density profiles of silk atoms along the x-axis disclosed that silk fibroin had a similar structure on GO and silicon dioxide surfaces at the initial stages of interaction, while in contrast, the same structure was disrupted on the graphene surface, especially those residues adjacent to the backbone tails.

4.4 Methods of preparation of CoNFs

After nearly half a century of research and development, CoNF isolation methods have gradually become mature. A variety of collagens, such as type I, II, III, IV, have been successfully extracted from different tissues, and some of them have been marketed [306]. On the other hand, another direction to produce the CoNFs is an assembly of the collagen molecules *in vitro*, that is, the reconstituted CoNFs. The assembly processes usually start with the solution of acid soluble type I collagen. These self-assembly processes are thermodynamically driven aggregation following neutralization of the solution and occurred spontaneously at neutral pH and physiological temperature[58]. Different fibrogenesis conditions, such as temperature, pH, and chemical reagents, have been employed to regulate the assembly process. In recent years, biotechnology scientists have also tried to use recombinant DNA (genetic engineering) technique to transfer the collagen-related genes into cattle, sheep or other livestock cells. The resultant collagen can be purified from the milk[58]. However, this technology is still in its infancy, and the industrial production has not yet been realized.

4.4.1 Top-down approaches—Collagen is usually extracted from collagen-rich tissues, such as skin, tendons, and bones. However, most of the collagens are insoluble in water due to the presence of the cross-linked network structures in collagen fibers.

Therefore, various processes, such as acid-, alkali-, salt-, or enzymatic-based treatments, have been developed [58, 307]. Accordingly, the ways to extract collagen can be classified as chemical- and enzymatic- methods [306]. The chemical method can be further divided into the basic-, neutral salt- and acid- method according to the different solvents that used for isolation. The chemical hydrolysis is more commonly used in industry, but enzymatic approaches are more promising when products required the high nutritional value and improved functionality. In a typical isolation processing (Figure 19) [308], the raw collagen materials, such as animal skin, scales, and bones, was firstly pretreated with acid or alkali and then extracted with acetic acid. After this processing, the acid-soluble collagen was obtained, and it was further digested with pepsin to product enzymatic soluble collagen. Different purification process, such as repeated dialysis, centrifugation, and another purification process, can be applied to purify this as-prepared acid-soluble and enzyme-soluble products [306]. Specifically, the route for collagen isolation can be divided into three steps, including pretreatments, collagen extraction and post-treatment.

4.4.1.1 Pretreatments: Different tissues are abundant with a different type of collagens. For instance, type I collagen are mainly found in skin, tendon, vascular ligature, organs, bone, while type II and type III are usually found in cartilage (a main collagenous component of cartilage) and reticulate (the main component of reticular fibers), respectively [54]. Meanwhile, even using same tissues, an optimized processing strategy also need to consider. For example, the collagen can be easily extracted from the calfskin, while is much difficult to obtain by using the cattle skin [60]. Accordingly, select the appropriate tissue material is the first step for the efficient extraction of CoNFs. Also, the non-collagen ingredients, such as minced meat and fat, *etc.*, need to be removed before the extraction processing. Further, some additional pretreatments are required, for example, decalcification, a process that treated of bone tissue by using 0.5 mol/L ethylenediaminetetraacetic acid buffer (pH 7.4, room temperature), was usually carried out to remove hydroxyapatite from bones. And a 1 mol/L Tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl) solution (pH 7.5) regularly was employed to wash against with the tissue at room temperature to remove of proteoglycan from cartilage tissue [60]. For other soft tissues, the soluble non-collagen material can be removed by using water and neutral salts, although some water-soluble collagen would also be removed in this processing. Alternatively, they can be treated with a 0.05 mol/L Tris-HCl buffer that contains 4.5 mol/L sodium chloride (NaCl) and moderate protein inhibitors. Under this condition, all collagens could not be dissolved while the other non-collagenous proteins were removed. Additionally, CoNFs dissolved very slowly in water and even in boiling water due to the cross-linked structures of the collagens in the connective tissue of animals, so a mild chemical treatment is necessary to break these cross-links before extraction [309]. To this end, diluted acids and bases have been employed, and the collagen was subjected to partial hydrolysis. After these process, the collagen chains remained intact, but the cross-links were cleaved [310].

4.4.1.2 Collagen extraction

4.4.1.2.1 Chemical methods: Both alkaline and neutral salts can be used for hydrolysis the CoNFs, but both have limitations [60]. Alkaline hydrolysis consists of treating the tissue materials with a basic solution, usually NaOH, for a few hours to several weeks. However,

alkali extraction usually only produces CoNF with low molecular weight, due to the hydrolysis of peptide bonds. The severe hydrolysis of collagen even leads to the production of the racemic mixtures composed of D-type and L-amino acids. Some D-type amino acids are toxic, and some have carcinogenic, teratogenic and mutagenic effects [311]. Therefore, CoNF produced by this method could not be used in biomedical fields. For salt extraction, neutral salts such as Tris-HCl, NaCl, citrate, are commonly used. Collagen is soluble in these neutral salt solutions when the concentration of salt reaches a threshold, but the impact of neutral salts on the dissolution of collagen is complicated. More importantly, cross-linked collagen molecules are complex in terms of their breakdown in such processing [59].

Acid hydrolysis methods mainly destroy the salt bonds between the collagen molecules and Schiff bonds, causing collagen fibers to swell and dissolve to achieve extraction [306]. Acid extraction of collagen can maximize the triple helical structure, and the resultant materials are suitable for biomedical applications. Therefore, acid hydrolysis is a more useful method for CoNF isolation, which can be performed by using organic acids (e.g., acetic acid, citric acid, and lactic acid) as well as inorganic acids (e.g., HCl). The organic acids extract CoNF more efficiently [312, 313], because they can dissolve the non-crosslinked collagens and also can destroy some of the inter-strand cross-links in collagen, leading to higher solubility of collagen during the extraction process [314]. In the extraction of acid-soluble collagen, pre-treated tissues were first dissolved into the acid solution, commonly 0.5 M acetic acid, and incubated at 4°C with constant stirring for 24–72 hours [315]. The difference in structure and physical properties of the CoNFs extracted from the inner layer of bovine hide under various temperature (50, 60 or 80°C) and pH (3, 5, 7 or 10) was reported [316]. Processing at a temperature of 80°C and a pH below the isoelectric point produced the highest level of soluble CoNFs. In contrast, extreme extraction conditions, such as at pH 3–10 or at 60–80°C, led to the denaturation of all the collagen with reduced molar mass. The effects of concentration of acetic acid (0.3, 0.5 and 0.8 M), temperature (10, 20 and 30°C) and extraction time (12, 24 and 36 hours) for the extraction of acid-soluble CoNF from the skin of grass carp (*Ctenopharyngodon idella*) was reported [313]. By increasing the temperature and concentration of acetic acid, the yield of CoNF increased, followed by a substantial reduction. Accordingly, the optimal protocol to harvest the acid-soluble collagen from the skin of grass carp was using acetic acid at a concentration of 0.54 M and incubated the solution at a temperature of ~25°C for ~32 hours.

4.4.1.2.1 Enzymatic hydrolysis: Compared with chemical methods, enzymatic processes feature rapid hydrolysis reactions and no environmental pollution during the extraction, and the hydrolyzed CoNFs exhibited high purity, water solubility, and stable physical/chemical properties. For enzymatic extraction, the raw tissue was added to 0.5 M acetic acid solution, which contained selected enzymes, such as pepsin, trypsin, and chymotrypsin [317]. The resulting mixture was continuously stirred for about 48 hours at 4°C followed by filtration [318]. The filtrate was precipitated and dialyzed under the same conditions as used for processing acid soluble collagen to obtain the final CoNF solution. In this process, the enzyme, such as pepsin, can catalyze the hydrolysis of the non-helical regions, and leads to the expansion of the $\alpha 1$ and $\alpha 2$ chains, so the final CoNFs still had triple-helix structures

with reduced antigenicity. To this end, the CoNFs produced by enzymatic hydrolysis are candidates for biomedical applications.

Animal skins, such as pigskin, for example, are one of most commonly used raw tissues for type I collagen extraction [319]. In this process, the skin was separated from the pigskin tissue, and the hair and the cuticle layer were removed, followed by a process to remove the fat and mashing with a winch. Then, the skin tissue, 2.5 g/L was added to the acetic acid solution (0.5 mol/L) that contained a moderate content of pepsin. The hydrolysis was carried out by continuous agitation and digestion for 48 hours, followed by an ultra-speed and low-temperature centrifugation to harvest the supernatant. The extraction of CoNF from the skin of yellowfin tuna (*Thunnus albacares*) was optimized [320]. Here, pre-treatment was first performed with NaOH (0.5 to 1.3 N) at 9°C for 12 to 36 hours to remove the non-collagenous protein. Subsequently, pepsin hydrolysis with the pepsin concentration of 0.6 to 1.4% (w/v) was carried out in HCl solution (pH 2.0) at 9°C for 12 to 36 hours.

4.4.1.3 Post-treatments: Pure CoNFs produced through chemical- and enzymatic hydrolysis have different molecular weights, while the main hydrolysates are similar, including peptides, collagen, and other proteins. Therefore, after hydrolysis, various post-treatments can be used to separate and purify the CoNFs. Salting-out, separation and purification are three critical steps for post treatments [60]. Salting-out is a process that precipitates CoNFs and remove non-collagen proteins by adding NaCl into the as-extracted CoNF solution [321]. Through step-by-step salting-out processing, different types of collagen can be separated to some extent. Salting out processing [60] requires different NaCl concentrations under neutral and acidic conditions. Under neutral conditions, the concentration of NaCl should reach 4.0 mol/L or 20%, while under acidic conditions, the concentration of NaCl only 2.0 mol/L or 10%. In addition, different types of the collagens have different solubilities at different NaCl concentrations. Under neutral conditions, only 10% of type I collagen is precipitated, while type III collagen is precipitated with 1.7 mol/L NaCl solution. Therefore, all types of collagens can be separated by using step-by-step salting-out processing. The precipitate in salting out is usually collected by centrifugation and further redissolved in 0.5 M acetic acid and dialyzed against 0.1 acetic acids for 2 days, and distilled water for 2 days [322]. However, for production of high purity collagen, protein purification techniques such as chromatographic separation and electrophoresis are required, although these processes increase costs and reduce yields [59].

4.4.2 Bottom-up methods—As with silk fibroin, collagen as a protein can assemble into CoNFs *in vitro*. Such self-assembly occurs spontaneously at neutral pH and physiologic temperature [58] and is a thermodynamic aggregation of collagen molecules. The CoNFs formed in the *in vitro* self-assembly process presented similar supramolecular structures to those observed *in vivo*, for example, they also have a characteristic “D” periodicity. However, the CoNFs vary in length and diameter according to the different self-assembly conditions. Similar to the kinetics of self-assembly of proteins such as silk [45] and amyloid fibrils [323], the kinetics of the growth of CoNFs can be divided into three phases [324]: (i) temperature-dependent lag phase where CoNF precursors, e.g., dimers and trimers are formed, (ii) temperature-independent growth phase where CoNF growth occurs, and (iii) a

temperature-dependent plateau phase where the self-assembly of CoNF is stable. During the self-assembly of CoNFs, the 5 linear trimers aggregated into intermediates, which added to the ends of CoNFs by linear growth [325]. As a result, the CoNFs grew linearly. This self-assembly processing and morphologies of the resultant CoNFs were highly dependent on the self-assembly conditions (temperature, buffer, and collagen concentration) [326, 327], source and type of collagen, as well as the extraction method. As an example, type I CoNFs could be reconstituted in vitro at neutral solution conditions with physiological temperature, where the monomers initially assembled into small fibrillar segments with weak interactions that were further enhanced by covalent cross-linking. These fibrillar segments then packed into long continuous CoNFs via end to end fusion, and assembled into a highly interconnected random network [58].

5. Applications of biopolymer nanofibrils

Biopolymer nanofibrils have mostly been investigated to reinforce polymer composites due to their unrivaled availability, low-cost, lightweight and remarkable strength and toughness. However, their applications have been widened into a variety of emerging fields in recent years, such as biomedicine, optoelectronics as well as energy & environments. In this section, we highlight the most recent studies of biopolymer nanofibrils related to these fields.

5.1 Applications of CNFs

In this decade, as a renewable and unique biopolymer nanomaterial, CNF has been widely used for the construction of optoelectronic, energy and environmental devices in both scientific and commercial communities. The estimated global production capacity of nanocellulose (including CNFs and CNCs) was on the order of 600 metric tonnes/year in 2013, and the future global market potential has been estimated to be 35 million metric tonnes/year [328].

5.1.1 Nanobuilding blocks in composites—In 1995, the utilization of CNCs as reinforcing fillers for nanocomposites due to their ultrahigh mechanical strength was first reported[329]. Since then, many innovative CNF/CNC-based nanocomposites were developed through various coordination hybridizing strategies [330–339]. Using tunicate CNCs as building blocks, a versatile approach to construct polymer nanocomposites was developed (Figure 20A–D) [340]. In this process, a CNC template was first formed by gelation through solvent exchange of CNF aqueous dispersion with a water-miscible solvent. Then, the gelled CNF scaffolds were imbibed with a polymer solution, which was miscible with the gel solvent but did not re-disperse the CNCs. Next, the composite gels were dried and compacted by compression molding, resulting in homogeneous CNC/polymer nanocomposites. Owing to the formation of a continuous CNC network within the nanocomposites, the shear moduli of the resultant nanocomposites increased dramatically from 1.3 MPa for the neat ethylene oxide–epichlorohydrin copolymer (EO-EPI) to 300 MPa for an optimal CNC/(EO-EPI) composite. Inspired by the sea cucumber dermis, which has the ability to rapidly and reversibly alter the stiffness of their inner dermis through dynamic regulation of nanofibril alignments, the stimuli-responsive polymer-CNC nanocomposites

were developed with a chemoresponsive mechanical adaptability (Figure 20E–G) [341]. These composites exhibited reversible reduction of the tensile modulus by a factor of 40 upon exposure to a chemical regulator, and more substantial modulus changes (4,200 to 1.6 MPa) upon exposure to emulated physiological conditions were realized by using a host polymer with a thermal transition in the regime of interest.

CNFs also are excellent raw materials for building functional aerogels/foams [342–347]. Freeze-dried BC aerogels were used as templates to prepare magnetic aerogels, which were further compacted into a stiff magnetic nanopaper (Figure 20H–J) [348]. The lightweight, highly-porous (porosity: 98%) and magnetic aerogels were prepared by using BC hydrogels (thickness of 20–70 nm) as templates for the non-agglomerated growth of ferromagnetic cobalt ferrite nanoparticles (40–120 nm in diameter). The aerogels could be actuated by a small household magnet and could absorb and release water periodically upon compression. By integration of wood CNFs with GO, thermally insulating and fire-retardant lightweight anisotropic foams were produced by freeze-casting of the hybrids consist of the CNF suspensions, GO, and sepiolite nanorods (Figure 20K–O) [349]. The foams displayed uniform and aligned tubular porous structures, and each component was homogeneously distributed within the walls without any aggregates. These ultralight foams showed excellent combustion resistance and exhibited a thermal conductivity of $15 \text{ mW m}^{-1} \text{ K}^{-1}$, which is around half that of expanded polystyrene. At 30°C and 85% relative humidity, the foams retained more than half of their initial strength.

5.1.2 Optical applications—The unique characteristics of CNFs, including the slender size (smaller than the wavelength of visible light) and high mechanical- and thermal-performance, endow the promising applications in optical fields. Transparent CNF films (usually termed as nanopapers) were produced when the CNFs were densely packed, and the intervals between the CNFs are small enough to avoid light scattering [350, 351]. Therefore, CNF-based optical materials had high optical transparency, excellent flexibility, and low thermal expansion. Compared with CNF films composed of CNFs with larger diameters, CNF films made of smaller diameter CNFs often have better optical transparency due to more dense packed CNFs, resulting in the low resistance for passage of visible light. In addition, CNF diameter and other parameters (such as orientation and degree of polymerization, porosity and moisture, and counterions) impact the mechanical properties of the CNF papers [352]. The 15 nm width wood CNFs were used to generate films which exhibited high transparency (71.6% transmittance at a wavelength of 600 nm), high mechanical performance (modulus of 13 GPa and strength of 223 MPa), and minimal thermal expansion (8.5 ppm K^{-1}) (Figure 21B) [353]. TEMPO-oxidized CNFs (3–4 nm in width) were used to fabricate transparent CNF films [186]. The films, with an approximately 20 μm in thickness, displayed high transparency with transmittance at 600 nm >75%. An optically transparent BC-based nanocomposite was also developed (Figure 21A) [206, 212, 354, 355]. In this system, the BC networks were filled entirely with the transparent resins. The addition of 70 wt% BC only resulted in the loss of 8% of the light transmittance than pure resins, and the composite displayed a low thermal-expansion coefficient (similar to that of silicon crystal) and a high mechanical strength. Also, the CNF-based materials can be

functionalized into organic light-emitting diode panels by deposition of electroluminescent layers on the transparent CNF-based substrates (Figure 21C and D) [206, 212, 351].

The rod-like CNC dispersions (suspensions) with concentrations of 3–7 wt% (or higher) can assemble into liquid crystalline structures that always exhibit left-handed chiral nematic behavior (Figure 21E) [356, 357]. When these CNC dispersions are dried, these chiral nematic textures can be preserved in the solid films, which can selectively reflect the polarized visual light that has a wavelength nearly matching the pitch (distance between two planes with the same CNC orientation) of the films. This effect is different from circularly polarized light reflected from a plane mirror, where the incident light has a phase shift on reflection of 180° and results in the altering of handedness [358, 359]. The reflected light from a nematic structure presents the original handedness (the rotation direction of the layers of the helicoid), which means only circularly polarized light of the same chirality (handedness) as the helicoidal structure is reflected, while the light with opposite chirality is transmitted. A series of optical materials with iridescence were developed by using nematic CNCs as templates [360–369]. In a typical process, the precursors, e.g., tetramethyl orthosilicate ($\text{Si}(\text{OMe})_4$) and polymers, *etc.*, were integrated into nematic CNC dispersions followed by evaporation of the solvent to form a uniform film. Then, the mesoporous films with iridescence were obtained after removal CNCs from the materials. A mesoporous silica film was produced through calcination of the thin chiral nematic silica/CNC composite film [360]. After the calcination, the chiral helical organization of CNC was replicated by the mesoporous structures in the silica. This chiral periodic construction resulted in the chiral reflectance of visual light, and the reflected wavelength was tuned across the entire visible spectrum and the near-infrared region (Figure 21F–H) [360]. Further, the mesoporous materials were functionalized to display various structural color via infiltration of the pores, modification of the surface, or introduction of hard templates [370, 371]. Another route to fabricate the mesoporous films is to remove the matrix of the composites, resulting in the formation of mesoporous photonic cellulose films. A supramolecular co-templating method was developed to yield mesoporous photonic cellulose films [368]. In this technique, chiral nematic composite films consisting of CNCs and urea–formaldehyde resin were synthesized by self-assembly of aqueous CNF dispersions in the presence of urea–formaldehyde precursors through solvent evaporation. After heat-curing and treatment with KOH to remove the urea–formaldehyde, iridescent and insoluble mesoporous photonic cellulose films were obtained and could be used as pressure sensors due to their rapid and reversible color changes upon swelling (Figure 21I–K).

5.1.3 Electronic applications—CNFs themselves are not electrically conductive, but they are quite convenient to combine with other electrically conductive elements to form electronic materials (Figure 22 and 23). A variety of conductive materials, such as silver nanowires, graphene and carbon nanotubes (CNTs), have been patterned or deposited on the CNF-derived nanopaper through vacuum filtration [372, 373]. In these conductive CNF nanopapers, the CNF layer acted as a transparent flexible substrate, while the conductive material networks offered the conductivity. Also, because the conductive networks were embedded in the surface layer, the resultant system was stable during deformation the CNF layers and provided constant electrical conductivity (Figure 22A–D). A conductive CNC/GO

nanopaper produced by the spin-assisted layer-by-layer assembly was reported [374], and it was transparent with the ultimate stress of 490 ± 30 MPa, Young's modulus of 59 ± 12 GPa and toughness of 3.9 ± 0.5 MJ m⁻³. These nanopapers with high reduced-GO contents showed high conductivities, around 5,000 S m⁻¹. Besides the applications as electric circuits, the CNF-based conductive systems have been assembled into nanopapers with piezoresistive characteristics (Figure 22E–J) [373]. Specifically, a free-standing and flexible CNF-(crumpled) graphene nanopaper was made and its mechanical strength was significantly improved as compared with the loosely packed pristine graphene film. Further, they were embedded in an elastomer matrix and yielded a highly-stretchable strain gauge with a stretchability of 100%, much larger than CNF-graphene membranes (6%).

CNFs can also be integrated with conductive materials to fabricate conductive aerogels [375, 376]. A conductive CNF aerogel was developed by deposition of the single-walled CNTs on CNF aerogel [377]. This conductive CNF aerogel could be further compacted to transparent, conductive, and flexible conducting membrane (90% specular transmittance at 550 nm and 300 Ω □⁻¹ sheet resistance with AuCl₃-salt doping). In addition, a lightweight CNF/CNT aerogel (aerogel with 75 wt% CNFs had a density of approximately 0.01 g cm⁻³) was prepared, which presented mechano-responsive conductivity (Figure 23A) [376]. A change of pressure of 0.1 bar induced a 10% relative change in resistance. Another strategy for developing electric aerogels are pyrolyzed CNF aerogels under an inert atmosphere and conversion into carbon aerogels. The pyrolyzation of BC aerogels to generate ultralight carbon aerogels was reported [378, 379]. The carbon aerogels exhibited a 3D network structure with interconnected carbon nanofibers 10–20 nm in diameter, which responded to the mechanical strength and the conductivity of the aerogels [379]. When this carbon aerogel was gradually compressed, the electrical resistance decreased nearly linearly with the compressive strain from 0 to 70%. A closed circuit experiment confirmed the continuous change of current with compressive strain and high reversibility of this carbon aerogel (Figure 23D and E). The stretchability of carbon aerogels can be further improved by infiltrating the carbon aerogels with the polymer resin. For example, polydimethylsiloxane/carbon aerogel composites [378] exhibited outstanding electromechanical stability that combined high stretchability (larger than 80%) and high electrical conductivity (0.20–0.41 S cm⁻¹). The resistance of these composites only increased ~10%, even after 1,000 stretching cycles with maximum strain of 80% (Figure 23F and G).

5.1.4 Energy storage and conversion applications—CNFs have attracted attention in energy storage and conversion fields; with applications in supercapacitors, lithium-ion batteries, lithium-sulfur batteries, sodium-ion batteries and solar cells (Figure 24, 25, and 26). Similar to the demands of CNFs in electronic devices, CNFs for energy-related use require combinations with active electrochemistry materials, such as CNTs[380, 381], graphene [382, 383] and conductive polymers[384–387]. Vacuum-filtration, solution mixture, in-situ polymerization, layer-by-layer self-assembly, oven-drying, freeze-drying, and supercritical drying have been utilized to fabricate these nanocomposites [380–387]. Covalently cross-linked CNF aerogels were used as scaffolds to deposit CNT electrodes and separator through layer-by-layer assembly [388]. The walls of the CNF aerogels were gradually thickened with a sequence deposition of first electrode, separator and second

electrode. A series of CNF-based supercapacitors, such as a reversibly compressible 3D supercapacitor and a 3D hybrid battery consisting of a copper hexacyanoferrate intercalating ion cathode and CNT anode, were fabricated. These devices showed stable operation without short-circuiting, and they were bendable, compressible and could be made with arbitrary formats. For example, the 3D supercapacitor showed stable operation over 400 cycles with a capacitance of 25 F g^{-1} and was functional even at up to 75% compression [388].

Furthermore, CNFs can integrate with active materials to develop film/mat-based energy storage devices [178, 389–393]. A hetero-layered mat battery was designed based on a unitized separator/electrode assembly system, which consisted of CNF separator membranes and electrodes comprised of solely the CNT-netted electrode active materials (Figure 24A and B) [392]. In such system, the CNF separator helped to achieve the tightly interlocked electrode/separator interface and endowed the shape flexibility and safety tolerance, while the stacked anode and cathode separator/electrode assembly ensured the excellent electrochemical performance. By assembling a CNF separator membrane with CNF/CNT-intermingled electrodes [393], a hetero-nanonet rechargeable paper battery was designed which exhibited ultrahigh energy density (226 Wh kg^{-1} per cell at 400 W kg^{-1} per cell) and presented origami-like foldability (Figure 24C and D).

Another strategy to produce CNF-based electrodes is pyrolyzation, which can convert the CNF into carbon materials. The resulting carbon materials possessed an ultralow apparent density, high specific surface area, and high electric conductivity [378, 394]. These carbon materials can be further functionalized by activating [395–397], heteroatom-doping [396, 398] or decoration with active materials [397, 399]. For example, BC foams have been employed to fabricate 3D carbon aerogels, which were further decorated by loading with MnO_2 (as positive electrode material) and doping of nitrogen (as negative electrode material, Figure 25A–D) [400]. The final supercapacitors could be reversibly charged/discharged at an operation voltage of 2.0 V in 1.0 M Na_2SO_4 aqueous electrolyte, delivering a high energy density of 32.91 Wh kg^{-1} and a maximum power density of $284.63 \text{ kW kg}^{-1}$. The devices also possessed good cycling stability with approximately 95.4% capacity remaining after 2000 continuous cycles (Figure 25E and F).

The CNF-derived carbon materials have also been developed into versatile electrodes for energy storage devices such as lithium-ion batteries [401], lithium-sulfur batteries [402–404] and sodium-ion batteries [405–407]. For example, BC-derived carbon aerogels were functionalized into anode materials for lithium-ion batteries via incorporation of SnO_2 and/or Ge nanoparticles. This hybrid material exhibited improved electrochemical performance, including high specific capacity and excellent cycling stability (Figure 25G–I) [401]. These hybrid systems still retained reversibility of approximately 600 mAh g^{-1} even after 100 cycles under a current density of 100 mA g^{-1} . This specific capacity was 2 times the theoretical capacity of graphite. In contrast, bare SnO_2 only delivered a particular capacity lower than 100 mAh g^{-1} . The outstanding performance of the anode was attributed to the well-dispersed active nanoparticles and interconnected conductive carbon nanofibers. These synergistic effects ensured electron conduction pathways were penetrating the whole electrode scale and numerous interconnected voids facilitated the diffusion of lithium ions.

The solar cell is another emerging field where CNF-based materials have been assessed [408–411]. CNF nanopaper had large forward light scattering, which increased the light path length, resulting in more light absorption in the active layer (Figure 26A) [409]. Optically transparent and electrically conductive nanopaper was developed by integration of CNFs with silver nanowires and further assembled into a solar cell, which exhibited a high-power conversion efficiency of 3.2% (Figure 26B) [410]. Due to the high affinity and high degree of entanglement between the CNFs and the silver nanowires, the transparent conductive nanopaper maintained its high conductivity and the solar cells still generated electrical power when folded and unfolded. The fabrication of solar cells on optically transparent CNC substrates was also reported (Figure 26C and D) [411]. This solar cell displayed proper rectification in the dark and reached a power conversion efficiency of 2.7% and could be easily separated and recycled using low-energy processes at room temperature.

5.1.5 Environmental applications—The entangled and packed CNF networks allowed CNF films to be used directly as filtration membranes for environmentally-related purification. CNF membranes have been used for filtration of nanoparticles (such as an inorganic nanoparticle, bacteria, and viruses) through size exclusion because the pore sizes in CNF membranes are smaller than the most of nanoparticles (Figure 27A and B) [412]. The pore size and porosities in the CNF membranes can be optimized through cross-linking [413], hot-pressing [414], and reducing membrane thickness [415]. CNFs can further integrate with other components, such as GO [416] and silk fibroin [417] to generate water purification membranes. The spontaneous formation of peculiar “shish-kebab” nanostructures with the periodic arrangement of silk fibroin domains along straight segments of CNFs was reported (Figure 27C and D) [417]. The formation of these “shish-kebab” nanostructures was facilitated by the preferential organization of heterogeneous (β -sheets and amorphous silk) domains along the CNFs, driven by the modulated axial distribution of crystalline planes, hydrogen bonding, and hydrophobic interactions. The nanoporous membranes allowed for high water flux, up to $3.5 \times 10^4 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$, combined with a high rejection rate for various organic molecules and metal ions (Figure 27E) [417].

CNF-derived materials have also been pursued oil/water separation. CNF-derived aerogels/foams are super-hydrophilic due to their high porosity as well as the existence of abundant hydroxyl groups. During the oil/water separation, water can quickly spread over the materials and autonomously permeate. After saturated absorption, the pores were filled with water, which was able to substantially reduce the contact area between oil droplets and the solid surface of the CNF-derived materials [418]. This effect acts as a repulsive cushion to the oil and enables oil/water separation. CNF/chitosan nanocomposite foams were used for oil/water separation [418]. The water flux for the composite foams reach $3.8 \text{ L m}^{-2} \text{ s}^{-1}$, and the whole separation process of a 300-mL mixture of kerosene/water (40% v/v) was completed within 60 s, with all of the oil rejected. CNF-derived carbon aerogels were also useful for adsorbing organic solvents and oils from water, due to the strong absorptivity and the hydrophobic features of the carbon [379, 394]. BC-derived carbon aerogels absorbed organic pollutants and oils with weights 106 to 312 times higher than the material weight (Figure 27F) [379]. CNF-derived carbon aerogels showed a remarkable capacity for the absorption of a variety of oils and organic solvents with weight gains ranging from 7,422 to

22,356 wt/wt (Figure 27G) [394]. Additionally, the carbon aerogels could be recycled through various approaches such as squeezing, combustion, and distillation. Due to the excellent thermal stability and robust mechanical properties, the carbon aerogels retained their superior absorption performance even under extreme conditions (e.g., severe temperatures and in corrosive liquids) [394, 419].

5.2 Applications of ChNFs

Different from CNF-based materials produced from top-down approaches, which normally have poor biocompatibility and biodegradability, ChNFs retained the beneficial characteristics of chitin and presented excellent biocompatibility, biodegradability, low immunogenicity and antibacterial activity [420–423]. Therefore, in addition to applying in optics, electronics, energy, and environment, ChNFs also widely used in biomedicine. In this section, we summarize these typical applications of ChNF-based materials.

5.2.1 Biomedical nanomaterials—ChNF-based materials have been widely used in tissue engineering, drug delivery, wound dressings, anti-cancer treatments, antimicrobial agents, reduction of obesity and biosensors [424–426], because ChNFs can be easily processed into different material formats, such as sponges, scaffolds, gels, and membranes.

5.2.1.1 Applications in tissue engineering: Tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences towards system designs. The area involves various aspects of living cells to process into biological substitutes for implantation into the body and/or to guide tissue formation in some active manner [425, 427]. In particular, the fabrication of materials with specific biological and mechanical properties that mimic the native extracellular matrix for regulating the cellular behavior is of interest in the field. ChNFs have attractive chemical, morphological and mechanical advantages for their applications in tissue engineering [428, 429]. Chemically, the ChNFs are similar to the glycosaminoglycans in the body; morphologically, they are similar to fibrous collagen structures in the native extracellular matrix [216, 430]; and mechanically, ChNFs produced from the exoskeleton of crustaceans have high strength (~90 MPa).

ChNFs have attracted attention in bone tissue engineering because they can be easily molded into different geometries for supporting the attachment and the proliferation of osteoblast cells. In bone tissue engineering, ChNFs are usually combined with calcium minerals to improve both biochemical and mechanical properties of the materials [431–433]. ChNF hydrogels have been used as templates for mineralization of calcium phosphate crystals [431]. The resultant mineralized scaffolds accelerated osteoblast differentiation in subcutaneous tissues of rats. The ChNF-based materials, microspheres, for example, can be functionalized by direct coating of hydroxyapatite crystals. (Figure 28A) [434]. This nanocomposite material presented well-organized hierarchical structure and promoted cell adhesion and osteoconduction and healing of bone defects *in vivo* (Figure 28B).

Besides bone tissue engineering application, ChNF-base materials have also used for hosting the other cell system. Compared to the commercial chitin microfibers, ChNFs with higher surface area promoted the cell adhesion and spreading of human keratinocytes and fibroblasts [435]. Additionally, ChNFs were combined with various synthetic polymers and

biopolymers, such as polyglycolide, polycaprolactone, polylactic acid, collagen, cellulose, silk and chitosan, to improve the strength and cell adhesion of the materials [203, 421, 436]. These studies demonstrated a potentially useful application of ChNFs in neural regeneration and vascular tissue engineering. Notably, for tissue engineering applications, the most used ChNFs are produced from top-down methods or electrospinning, recently, new types of ChNF microspheres were fabricated through self-assembly of chitin molecules in a NaOH/urea aqueous solution [437]. These ChNF microspheres supported the human hepatic cell line L02, where cells adhered and covered the ChNF microsphere surfaces, can be directly used as cell carriers.

5.2.1.2 Applications in wound dressing: The ChNFs have been processed into different wound healing materials, due to their properties such as gas permeation, high porosity, and high specific surface area [438]. Stability and antibacterial properties are two critical factors to evaluate the performance of the wound healing materials. ChNFs membranes were resistant to digestion by egg lysozyme treatment, while slightly degraded when treated with cellulase [439]. Deacetylated ChNFs can partially inhibit the growth of *E. coli*, with inhibition due to the positive charges on the surface of membranes. Superficially deacetylated ChNFs promoted re-epithelization and proliferation of fibroblasts, which induced the proliferation and re-modeling phases in wound healing [426]. Cell-encapsulated ChNF-based hydrogels induced the differentiation of bone marrow mesenchymal stem cells (BMSCs) in the absence of inducers [440]. This ChNF-based hydrogel protected BMSCs from elimination by the harsh wound microenvironment (Figure 28C). The effectiveness of a spray, a gel and a gauze prepared with ChNFs in association with chitosan for healing cutaneous lesions was assessed [220, 424]. Also, an advanced sterile wound matrix, Talymed® (Marine Polymer Tech., MA, USA), has been manufactured using ChNFs produced from marine diatoms, where the ChNFs have an average length of 4–7 µm and a width of 100–150 nm. The tolerability, safety, and efficacy of Talymed® was demonstrated for the treatment of patients with leg ulcers over 3 months [441].

5.2.1.3 Applications in drug delivery and oral administration: Water-soluble chitin/chitosan has been intensively applied for drug delivery systems [422], however, for these systems, how to improve the specific surface area and the bioavailability of the vehicles remains a big challenge. More recently, the electrospun nanofibrils were used to fabricate materials with higher drug encapsulation efficiency. The drug release behavior can be easily regulated by controlling the composition, morphology and porosity of the vehicles [432, 442, 443]. Through the creation of diffusion pathway and/or degradation of polymers, these vehicles can release various therapeutic agents, such as proteins, growth factors, antibiotics and specific drugs [425]. Azuma et al. [444] evaluated the preventive effects of ChNFs in a mouse model of dextran sulfate sodium-induced acute ulcerative colitis. The results indicated that ChNFs proved a significantly reduced disease activity index (Figure 28D–F), which conversed to the chitin powder that did not suppress dextran sulfate sodium-induced acute ulcerative colitis. Furthermore, ChNFs also could inhibit mucosal inflammation by suppressing the myeloperoxidase-positive cells (Figure 28G–I). In addition, the oral administration of ChNFs in animal tests has been recognized as emerging in the areas of improved intestinal health, ameliorates renal injury and reduction of obesity [444, 445].

Carefully examination of previous works of the ChNF-based drug delivery system can found that most of these studies were focus on using ChNF produced from bottom-up methods, while top-down produced ChNFs are lack for these applications, which is a future direction that worth for address, in particular, because of the chitin and its derivatives has nonspecific antiviral and antitumor activities, and the size of chitin alter its effects on immune cells [216–219].

5.2.1.3 Applications in biosensors and diagnosis: Biosensors are platforms for sensitive and diagnostic assessments based on molecular biological recognition elements (such as enzymes, antibodies, receptors, and single-stranded DNA) which display specificity and affinity for their substrates or binding partners [446, 447]. Chitin requires harsh conditions for complete dissolution and processing, which limited the utilization of chitin in biosensors, although this issue can be partially solved by using ChNF dispersions[216, 220, 432, 442, 448]. In comparison, chitosan, a chitin derivative, is somewhat soluble in acidic aqueous media and can be processed to nanofibrils under various conditions [424, 425]. Chitosan nanofibrils have been used as substrates to hold other components, such as gold nanoparticles [449], zinc sulphide (ZnS)/folic acid [450] and poly(vinyl alcohol) [451] for fabrication of biosensors. Similarly, reinforced chitosan beads prepared from partially deacetylated α -ChNF aqueous dispersions were applied for the immobilization of β -glucosidase [247]. The 1/5 deacetylated/ α -ChNF composite beads presented significantly improvement in the immobilization efficiency of β -glucosidase (67%) (Figure 28J), compared to the pure chitosan beads (40%) (Figure 28K). The deacetylated/ α -ChNF composite beads had higher porosity and better mechanical properties, which was also confirmed by Brunauer–Emmett–Teller analysis and rheology.

5.2.2 Optical applications—ChNFs membranes are generally optically transparent even with a high fiber content due to the fine width of the ChNFs (approximately 3–100 nm) [452]. Transparent ChNF membranes were prepared by coagulating chitin solution (where the chitin was dissolved in 11 wt% NaOH-4 wt% urea aqueous solvent at low temperature) with EtOH [453]. These robust ChNF membranes presented homogeneous mesoporous structures and excellent gas barrier properties (0.003 bar for oxygen permeability). Therefore, these materials were considered good candidates for food and medical packaging materials. Composite ChNFs with eleven types of acrylic resins were used to prepare thin membranes, and their thermal expansion, transparency and mechanical properties were evaluated (Figure 29A) [230]. All the composite membranes had high transparency, even when the fiber content was as high as 40 wt% and the thickness of membrane was 60 μ m, offering a simple approach to produce transparent composites, and the ChNFs reinforced the mechanical properties of the composite materials. A one-step approach for the fabrication of self-assembled ChNFs structures with features ranging from micrometers to approximately 50 nm was reported[264]. This technique was achieved by the integration of the ChNF ink with airbrushing and soft lithography (replica molding and microcontact printing). The volatility of the ChNF ink afforded ambient chitin patterning in one step, a distinct advantage compared to aqueous-based self-assembled nanofibril or hydrogel systems. The formed ChNFs templates, as diffraction gratings, provided a proof-of-concept for ChNF-based biophotonics devices (Figure 29B).

5.2.3 Electric and energy applications—Chitin is known as the most abundant natural nitrogen-containing biomacromolecule. Thus, chitin could be transformed into N-doped carbon-based materials and applied as electrocatalysts for oxygen reduction reactions [454]. Native ChNF materials from prawn shell were used to synthesize porous aerogels via a chemical route, a process that treated by KOH, HCl and NaCl, to remove the other components in the shell (Figure 29C) [455]. Thereafter the aerogel was carbonized at 900 °C to obtain porous N-doped carbon materials with a surface area of 526 m² g⁻¹ and an N doping level of 5.9%. These ChNF based N-doped carbon materials had more positive oxygen reduction reaction onset-potential, better stability and high resistance to the methanol crossover effect (a phenomenon by which methanol diffuses through the membrane without reacting).

Two dimension-nanosheet material were prepared by hydrophobization-induced interfacial-assembly of native ChNFs [456]. These amphipathic chitin nanosheets were water-dispersible and had excellent emulsifying ability, where Pickering emulsions with internal phase were up to 83.4%, and droplet size was up to 140 μm [456]. After the emulsifying and carbonization process, the ChNF-based precursors were converted to carbon nanosheets. After that, these carbon nanosheets were filtered with chitin suspensions to form an all-chitin derived flexible electric circuit. These hybrid circuits with the thickness of the carbon layer approximately 1 μm (Figure 29D) were stable to twisting, folding, and peeling with tape. These carbon nanosheets provided a new option to exploit bio-based electric devices. ChNF nanopapers were fabricated by a centrifugal casting technique and had an optical transmittance of approximately 92%, Young's modulus of 4.3 GPa, a coefficient of thermal expansion of around 17 ppm K⁻¹ without a glass transition temperature [452]. The same group further fabricated an organic light-emitting diodes device on their ChNF nanopapers to demonstrate the potential of ChNF nanopapers as a platform material for flexible green electronics (Figure 29E) [452].

5.2.4 Environmentally-related nanomaterials—The ChNFs prepared by mechanical treatment were applied to produce optically transparent composite nanofiber sheets with acrylic resin [457]. During the filtration process for producing the nanofibril sheets (90 mm in diameter), the ChNFs had a faster sheet-formation speed than CNFs [457], when the content of water suspension of CNFs and ChNFs was fixed as 0.1wt %. This situation was proposed to be due to the replacement of hydrophobic acetamide groups on the C₂ position of ChNFs, resulting in ChNFs that were less dispersed in water than CNFs. On the other hand, the ChNFs and their derivatives have fascinating adsorption performance due to the abundant active functional groups, such as primary amino and/or hydroxyl groups as well as acetamido groups [458]. Therefore, ChNF-based materials were recently employed as filtration materials for water purification. Ultrafine ChNFs with diameters of 5–10 nm were used to fabricate barrier layers in composite membranes for ultrafiltration of bilge water [459]. After 2 days of filtration at 30 psi, the permeation flux of ChNF-based composite membranes was 217.0 L/m² h, which was 8–10 times higher than that of commercial polyacrylonitrile 10 (Sepro, California), and the rejection ratios were maintained above 99.7% in whole filtration processing (based on the total organic carbon analysis) (Figure 30A).

Chitin and chitosan-derivatives with high contents of functional groups have gained wide attention as effective bio-sorbents for the removal of various pollutants [458, 460, 461]. For example, ChNCs can stabilize the corn oil-in-water (o/w) emulsions, which show about one-month stabilization with the aid of ChNCs, suggesting that ChNCs can adsorb oil molecules among the interfaces of oil/water emulsion [462]. Chitosan nanofibril-based materials have also demonstrated abilities in adsorbing multiple cations, anions, and radionuclides in both air and aquatic environments pollutants [458, 460, 461]. Recently, the gas phase coagulation approach was employed to fabricate robust, self-standing, ChNF hydrogels with ultralow mass content (0.2 wt.%) [241]. ChNFs with different surface charges were treated with various gas phase coagulation baths to form homogeneous hydrogels, where the positively charged, partially deacetylated, α -ChNFs were treated with ammonia, and the negatively charged TEMPO-oxide ChNFs were treated with hydrogen chloride. The resultant hydrogels exhibited removal of dyes regulated by their surface charge (Figure 30B and C) [241]. Moreover, this work also demonstrated that ChNF-based hydrogels presented pH-sensitive swelling at an oppositely environmental charge. For instance, an increase of pH from 2 to 10 resulted in changes in the degree of swelling (the times of dry weight) of positively charged ChNF-based hydrogels, with a decrease from 268 to 130, while the negatively charged ChNF hydrogels increased from 128 to 242. These results suggested that the ChNF-based hydrogels with unique surface electrical properties were suitable as bio-sorbents, as well as bio-carriers for environmentally-related applications.

5.3 Applications of SNFs

5.3.1 Biomedical applications—Silk fibroin has been widely studied in biomedical fields due to its biocompatibility, tunable biodegradability, and versatile processability [463]. However, SNFs have several additional features for biomedical applications compared to silk fibroin. SNF hydrogels and scaffolds usually exist in hierarchical micro- and nanoporous structures, which are useful for cell adhesion/migration and nutrient transmission [276]. Furthermore, the pore sizes and porosity of SNF materials are tunable from several nanometers to hundreds of micrometers by controlling the concentration of SNFs or adding sacrificial components (e.g., water-soluble salts [464, 465] and polymers [466], and enzymatically degradable amyloid fibrils [267]). Previous reports indicated that the pore size of SNF materials could be controlled to the several nanometer scale range due to the nanosize effect and high specific surface area of single nanofibrils [267, 280]. Further, as protein nanofibrils, SNFs have a similar structure (3–5 nm in diameter and several micrometers in length) and mechanical properties (modulus around 2.5 GPa) to CoNFs in biological tissues [467]. Mechanical rigidity can be further enhanced through water-annealing [21, 468] or with EtOH [45, 47], which are two approaches used for inducing the conformational transition of silk fibroin from random coil/ α helix to β -sheet. These post-treatments do not impact the topographic structures of the final materials [297]. These advantages allow SNF applications in the construction of *in vitro* extracellular matrix-like scaffolds for tissues, such as hydrogels and sponges. These biomimetic extracellular matrix materials have been used to regulate migration, proliferation, and the differentiation of the neural stem cells [297].

The random distribution of SNFs in scaffolds makes it more difficult to tailor cell alignment, which is the essential for many tissue and organ functions. Accordingly, various approaches have been explored for the regulation of SNF alignment in scaffolds, including layer-by-layer assembly [469], dry-spinning [115], electric fields [470] and tensile induced alignment [296]. Through the successive deposition of chitosan and silk fibroin on substrates, highly-aligned SNF layers were obtained due to electrostatically induced self-assembly [469], and this layer-by-layer assembly technique is suitable for producing thin films. Fiber-spinning techniques, including electronic spinning [275], wet-spinning [471, 472], dry-spinning [115, 473, 474], and microfluidic-assisted spinning [475, 476] are other useful approaches to regulate nanofibril orientation. The alignment of nanofibrils can be achieved by applying shear forces and post-drawing methods during spinning.

Dispersions or gels consisting of CNFs and ChNFs, have been used for spinning highly-oriented regenerated fibers [474–476], while silk fibril-based spinning has only recently been reported since methods to produce highly concentrated silk fibril spinning dopes were previously limited. SNF dispersions produced from liquid exfoliation (top-down) techniques, such as formic acid/ CaCl_2 [275] and HFIP dissolution [115], were spun into highly-oriented silk fibril-based regenerated fibers. The resultant fibers had diameters several micrometers to hundreds of micrometers and were several meters in length [115]. Remarkably, these silk microfibril dopes could be used for dry-spinning (Figure 31A). In such processing, the solidification of the fiber occurs due to the evaporation of volatile solvent into the air without any additional immobilization or post-processing steps. Thus, besides control of SNF alignment along the fiber axis, dry-spinning can generate a polymorphic arrangement of biopolymer nanofibrils in a single fiber (Figure 31B–D). For instance, yarn-like spiral fibers were produced by rotating the collector on a plane perpendicular to the fiber axis (Figure 31D) [115]. These highly-oriented hydrogels, thin films, and fibers supported stem cell growth along the direction parallel to fibrillar orientation (Figure 31E). Such macroscopically aligned constructs may be suitable to generate highly aligned tissues, such as in muscle fibers, spinal cord, and tendons. Many organs and tissues, such as blood vessels, intestine, and esophagus, are characterized by hierarchically arranged morphologies.

Electrostatic interaction [470] is another approach (Figure 31F) to drive the hierarchical alignment of nanofibrils to generate tissue scaffolds with anisotropic structure. In such a process, the orientation of nanofibrils is triggered by the electrostatic interaction of pH-induced electric dipoles of SNFs. In an electric field, the SNFs are entangled to adapt to the electronic polarity direction (Figure 31G), and the inherent repulsion from negative charge of each nanofibril results in the separation of the aligned layers and the formation of hierarchical anisotropic structures (Figure 31H). Mesenchymal stem cells showed oriented responses on the hydrogels, suggesting future applications in different tissue regeneration strategies where isotropy is a key, such as in nerve and muscle regeneration (Figure 31I). Combinations of spatial confinement and external tension were also a strategy for assembling SNFs into macroscopic hierarchical materials [296]. As shown in Figure 31J–N, a pre-designed mold can be used to tailor the orientation and gradients of SNFs in materials through shear flow and force extension during the gelation. This method also can generate complex hierarchical one- to three- dimensional architectures and enable programmable dynamic responses to humidity. For example, a one-dimensional actuator (Figure 31K) and

two-dimensional web constructs (Figure 31L–N) with fibrillar gradient alignments can be produced by introduction of buckling instabilities through anchor placement. This fibrillar orientation strategy offers a new route to designing biomedical materials with predicted structures and specified mechanical and physical properties.

Other interesting biomedical applications of SNFs are in developing injectable hydrogels [272, 276, 279, 477] for cell therapies, tissue engineering, and regenerative medicine [478, 479]. Compared with traditional hydrogels, injectable hydrogels can fill tissue defects with arbitrary shapes while minimizing surgical wounds and patient discomfort [480]. Drugs and cells can be incorporated into the hydrogels before injection through solution-mixing [481, 482]. Additionally, the 3D hydrous networks in hydrogels have ultrahigh permeability for nutrients, metabolites, and intercellular chemical signaling molecules [483, 484]. However, the production of injectable hydrogels combines the benefits of processing, structure and function remain a significant challenge. Like other hydrogels, the processing to obtain injectable hydrogels can be divided into chemical physical and gelation options [485, 486]. Among these, physical gelation is usually triggered by pH, ions, and temperature [485]. Thus, this approach is more green and straightforward than chemical gelation, which frequently requires cytotoxic solvents and harsh chemical reactions for molecular cross-linking. However, the mechanical properties of the physical hydrogels are weaker than the chemical crosslinked hydrogels. Also, integration of appropriate gelation rate and injection times in an injectable hydrogel system remains challenging [485, 486]. Many chemical and physical injectable hydrogels have long gelation times and are still flowable after injecting into tissue, which leads to unwanted leakage away from the target repair site.

SNF suspensions in high concentration are typical non-Newtonian viscoelastic liquids with shear-thinning characteristics, where viscosity decreases significantly with shear force [276]. Thus, these suspensions can be further converted to injectable hydrogels through simple processes such as centrifugation [276], horseradish peroxidase (HRP) crosslinking [279], and cyclic concentration–dilution processing [272]. The resultant injectable hydrogels displayed outstanding thixotropic capacity. For example, the storage modulus of SNF hydrogels produced from 7 vol% EtOH treatment could recover to 93% of the original value within 40 seconds after undergoing a significant shear strain of 5,000% (Figure 32A) [276]. This rheological characteristic allows injection of the hydrogels to the desired pattern followed by solidification *in situ*. These SNF hydrogels have successfully served as cell and anticancer drug loading carriers for sustained site-specific delivery (Figure 32B). Recent *in vitro* and *in vivo* studies revealed that doxorubicin (chemotherapy medication used to treat cancer) loaded silk hydrogels had an excellent antitumor response, outperforming the equivalent dose of free doxorubicin administered intravenously [279]. Most recently, laponite nanoplatelet and bioactive clay that can promote osteoblast growth, has been used to regulate the formation of silk fibroin hydrogels through control of the self-assembly of silk fibroin into nanofibrils (Figure 32C) [477]. During the silk fibroin gelation, laponite nanoplatelets served as a medium to accelerate hydrophobic interactions among the silk fibroin molecules and as a disruptor to limit the growth of β -sheet domains. The resultant nanocomposite hydrogels were injectable with thixotropy at room temperature. Osteoblasts encapsulated in the gels proliferated and enhanced osteogenic outcomes. Compared with pristine silk fibroin hydrogels, transcript levels for alkaline phosphatase, osteocalcin,

osteopontin, and collagen type I osteogenic markers in the nanocomposite hydrogels improved significantly over 14 days, especially for the hydrogels with a high content of laponite nanoplatelets.

Other merits of SNFs further illuminate silk material applications in biomedicine. For example, SNF dispersions can be stabilized in aqueous solutions for more than several months [267] and can be smoothly blended with other water-dispersible functional nanomaterials, such as GO [299], quantum dots [273], golden/silver/ferriiferous oxide nanoparticles and wires [267, 272, 273, 487]. Thus, these are useful for developing functional biomedical nanocomposites. Furthermore, SNFs have been used as initiators and nano-templates to control *in-vitro* mineralization [272, 281, 488]. The biominerals (e.g., calcium carbonate[272] and hydroxyapatite nanocrystals [281, 488]) or inorganic nanomaterials (e.g., silver and gold nanoparticles [272, 273, 487, 489]) that formed were trapped in the SNF networks and thus stabilized as a suspension in solution. This state contrasts with aggregates and sediments observed in more traditional silk fibroin-based hybrid systems. These well-dispersed SNF/biomineral systems are useful in materials formation, in part due to the biological-based components, combined with the robust mechanical performance of the final materials.

5.3.2 Nanotemplates for inorganic nanomaterials synthesis—Protein/peptide nanofibrils regulate mineralization as a fundamental strategy to build hierarchical structures in both living systems and novel advanced materials for bionanotechnology purposes [490, 491]. Protein/peptide nanofibrils are widely-used metal ion capping agents, which have a substantial capacity to capture metal ions through chelation and electrostatic interactions [273]. Moreover, protein/peptide nanofibrils themselves are reducing agents and can directly react with the metal ions in aqueous solution [281, 492]. These reactions are carried out at room temperature without high energy consumption, thus are much milder, green and cost-saving than the traditional sol-gel chemical reactions [493]. This route is in contrast with more conventional approaches, which usually require complicated synthetic process and use potentially harmful reagents, such as sodium borohydride (reducing agent), phenylethanethiol (protective agent) and tetrahydrofuran (solvent). Accordingly, the protein/peptide nanofibrils provide unique nano-templates for inorganic nanomaterial growth.

Hydroxyapatite nanocrystals [281, 488], calcium carbonate nanocrystals [272], ferrosiferic oxide nanoparticles[267], silver nanoparticles[272] as well as gold nanoparticles/platelets [273, 487, 489] have been synthesized by using SNFs as the reductant and/or the nano-templates (Figure 33). In the synthesis of gold nanomaterials, the reactions can be conducted: (i) at room temperature with illumination from the sun [281], or (ii) at high-temperature incubation (e.g., 80°C) [273], or with the addition of another reductant, e.g., sodium borohydride (NaBH₄) [487]. However, these methods usually share some common features. For instance, all of these *in-vitro* mineralization reactions can be considered as one-step synthesis processes, termed one-pot synthesis [494]. These reactions are typically carried out in aqueous solution, and the synthetic nanomaterials can be tightly bound to the nanofibril surface, trapped and stabilized by the nanofibril networks. Also, the size and shape of resultant nanomaterials can be regulated by controlling the pH and/or incubation time. Integration of inorganic nanomaterials with the SNF system further extends the

application of SNF materials due to the additive functions of the inorganic nanocomponents, such as magnetism from ferrosioferric oxide [267], conductivity of gold [487] [492], the antibacterial property of silver [495], as well as the excellent biocompatibility of calcium nanominerals [496]. These *in-situ* mineralized SNF nanocomposites have shown promising applications in ultrafiltration [281, 489], tissue regeneration [488, 497], conductive sensors [273, 489], and antibacterial materials [495].

5.3.3 Optical and electronic applications—SNFs typically exist as suspensions or hydrogels which contain a high water content (usually above 95 wt%). Thus membranes obtained from solution-casting tend to be brittle because of the low solids content and the numerous defects generated by the drying process. Fortunately, SNFs have long contour lengths and high mechanical strength and can withstand vacuum-filtration drying [267, 273, 281, 489]. Accordingly, vacuum-filtration is the most useful approach to fabricate homogeneous SNF membranes. Figure 34A presents a typical vacuum-filtration system used for membrane formation, which consists of three units: collecting liquid bottle, porous sand core filter, and cylinder funnel. These components are connected sequentially from the bottom to the top and sealed by rubber plugs. Before applying the vacuum, a filtration membrane with pore size of ~200 nm was mounted on the porous plates followed by adding the SNF solution. This vacuum filtration approach has been widely applied for making the other nanocomposite membranes, such as amyloid fibril membranes [498, 499], GO paper [299, 500, 501], and CNT films [502]. Previous works [267, 273, 281, 299, 489, 500, 501] confirmed the improved mechanical performance (strength, stiffness, toughness) of the membranes produced from vacuum filtration as superior to those produced by solution-casting.

The unique features of SNF membranes (produced from both top-down and bottom-up methods) are the high transparency (Figure 34B and E) and birefringence under polarized light (Figure 34F), because their pore sizes (Figure 34C, D, G) are typically smaller than the wavelength of visible light [267, 273]. For example, a ~200 μm thick SNF membrane is transparent in the visible region (300–800 nm) with transmission above 70%, and reaches up to 88% at 800 nm [273]. These transmission values are comparable to poly(methyl methacrylate) (92%) and polycarbonate (89%) films [508], which are two of the most widely used transparent polymer films. Also, SNFs can absorb dyes and quantum dots to generate membranes with different colors (Figure 34H) and to functionalize bioelectronic devices through patterning of the conductive layers (Figure 34I–K). These bioelectronic membranes with nonporous structures can trap and transmit water molecules at high environmental humidity, and hence can be adhered to gloves, skin and deform with skin deformation, suggesting utility for humidity sensors and electronic skins [273, 489].

5.3.4 Environmental applications—In addition, SNF membranes can be functionalized before membrane formation. The design and preparation of SNF based actuators with dual magnetic and water responsiveness is an example, [267] positively charged magnetic nanoparticles could be added into SNF dispersions before vacuum filtration (Figure 34L). In the hybrid suspensions, magnetic nanoparticles electrostatically attached to the nanofibrils and distributed homogeneously in solution, and thus can be

directly used for the generation of robust bio-actuators. In such nanocomposite systems, the magnetic response was received by the magnetic nanoparticles, while the humidity response was achieved by the protein nanofibrils, which can capture and hold the water in the membrane and alter the mechanical properties of the membrane from brittle to ductile (Figure 34L). Accordingly, when applying a magnetic field on the wet spline, it bent to a crescent shape and fixed its shape during drying, exhibiting a reversible shape-memory effect upon exposure to water in a cyclic process (Figure 34M).

Another promising application of the SNF membranes is ultrafiltration for water purification [267, 280, 281, 489]. Ultrafiltration membranes have several advantages for water purification, such as low-cost, high separation efficiency, and energy- and waste-efficiency [503–505]. However, available commercial ultrafiltration membranes, prepared from polysulfone, polyamide, and polyethersulfone, usually have poor water fluxes and poor solvent resistance [506–508]. A series of inorganic and polymer material system has been utilized to address these issues, including polystyrene and gold nanoparticles [509, 510], inorganic nanowires and nanofibers [511], GO [512], tungsten disulfide [513] and molybdenum disulfide [514]. However, the challenge remains to prepare low-cost and ultrathin filtration membranes that integrate high-water flux, mechanical strength, and chemical resistance, along with excellent separation performance.

The advantages of the SNFs for ultrafiltration applications have been demonstrated [267, 280, 281] (Figure 35A). The SNFs could be assembled into free-standing ultrathin membranes (e.g., the thickness of 47 nm) with highly porous structures and mechanical flexibility [280]. The pore size of the membranes could be controlled in a narrow range, e.g., 6–12 nm. In these pore size ranges, water pollutants such as dyes, proteins, and colloids of nanoparticles were efficiently rejected while the water passed through the membrane efficiently (Figure 35B). As a result, these pristine SNF membranes presented high pollutant removal efficiency and maintained ultrahigh water flux. For instance, a 40-nm thick membrane rejected rhodamine B efficiently with at least a 64% rejection, and water fluxes reached that of pure water fluxes of $13,000 \text{ l h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$, several times higher than most of other reported advanced filtration membranes.

As with other ultrafiltration membranes, possible shortcomings of the pristine SNF membranes are the inability to tune pore sizes, so the permeation rates of the water and other solvents declined dramatically with an increase in membrane thickness. Moreover, contaminants can clog the nanochannels due to the small pore sizes of the membrane. Thus molecule-loading capacity is limited. These problems can be solved to some extent by adding second components within the same membrane. For example, amyloid fibrils have been incorporated into SNF dispersions to obtain binary protein nanoporous membranes (Figure 35C) [267]. The amyloid fibrils were removed selectively using enzymatic etching since the amyloid fibrils are assembled from the enzymes that extracted from the eggs or milk and can be digested by pepsin, while silk is not susceptible to this enzyme. After this process, the pore sizes of the SNF membranes were increased ten-fold compared to the pristine SNF membranes. Besides the regulation of pore size, second components, such as CNFs [417] and Kevlar nanofibrils [489], have been utilized to improve the mechanical strength and chemical resistance of the membranes.

In contrast to homogenous nanoporous membranes, multilayer nanoporous membranes have unique advantages, including enhanced throughput, low-pressure drop, high molecule loading capacity, as well as high filtration efficiency [515–519]. Accordingly, several laminar inorganic nanosheets (e.g., GO[512], tungsten disulphide [513], molybdenum disulphide [514]) have been pursued to construct multi-layered filtration membranes. However, these membranes do not have nanoporous structures. Water and other solvents can only permeate through gaps and interlayer space between the inorganic nanosheets, and thus these membranes have low water-fluxes as well as low molecule loading capability. The synergistic integration of computational modeling and experimental fabrication provide a novel route to realize the *de novo* design of multilayer nanoporous membranes [281]. The design of nacre-like protein nanofibril/nanomineral nanoporous membranes was pursued, for example, where computational simulations based on coarse-grained models of protein nanofibrils and mineral nanoplates revealed that multilayer construction can only form with weak interactions between nanofibrils and mineral plates. By increasing the interfacial energy (γ) from 0 to 0.53, the two components trended to assemble into a more uniform structure (Figure 35D). This prediction process accelerated material screening and saved time and cost. Based on validation of the computational predictions, SNF and hydroxyapatite systems were chosen for experimental fabrication. The resultant SNF/hydroxyapatite membranes were prepared from protein self-assembly and in-situ biomineralization to attain highly ordered nano-sized multilayer structures (Figure 35E). The layer-distances were tuneable by controlling the weight ratios between the SNFs and hydroxyapatite. Profiting from these well-organized hierarchical multilayer nanoporous structures, these membranes showed ultrafast water penetration (over five times greater than other reported ultrathin membranes with similar thickness), and also exhibited universal and high efficiency to remove and even reuse (in some case) heavy metal ions, dyes, proteins, and nanoparticles.

5.4 Applications of CoNFs

The excellent biological properties, such as high biocompatibility, biodegradability, weak antigenicity[520, 521], made CoNFs as one of the most attractive materials for biomedical applications. But more than that, CoNFs have also gained considerable interests in energy, environmental, food, and cosmetic applications due to their abundant sources, hydrophilicity as well as high mechanical performance. In this section, we review the CoNF and collagen applications in these abundant fields.

5.4.1 Biomedical applications

5.4.1.1 Applications in Tissue engineering: CoNFs were processed into various formats, including sponges, hydrogels, powders, fibers, tubes, and membranes, *etc.*, to match the requirements for biomedical applications [520, 522]. One of most abundant biomedical applications of CoNFs is for cartilage repair, which is a challenging task, because of the non-vascularized tissues leading to a poor intrinsic regeneration capability of cartilage [523, 524]. CoNFs have the ability to provide favorable environments to maintain the proliferation and chondrification of mesenchymal stem cells [525]. CoNF-based sponges and hydrogels colonized with cells have been used to mimic cartilage structure and to regenerate cartilage in vitro (Figure 36A) [526]. Porous CoNF-based scaffolds and hydrogels with

interconnected structures promoted gene expression of cartilaginous matrix proteins in chondrocytes and supported and maintained chondrogenic stimulation of human mesenchymal stem cells [527]. The size and orientation of CoNFs are critical in influencing the biochemical properties and function of organism tissues [528]. For example, the stretching and recovering of tendons are attributed to the highly oriented CoNFs (with a length of microscale) (Figure 36B) [529]; the mechanical flexibility and optical transparency of corneal tissues were contributed by the anisotropic-stacked lamellar CoNFs (Figure 36C) [530]; and the superior fracture resistant of bones were ascribed to the highly organized CoNF/HAP layers [531] (as mentioned in section 2.4). Therefore, to mimic native tendon, cornea and bone structures, aligned CoNF materials have been used as structural supports for tendon regeneration [532, 533]; orthogonally disposed lamellar-stacked CoNFs have been applied for corneal stroma reconstruction [534]; and the nanostructured and mineralized CoNFs were fabricated to various shapes to serve as a prototype model for bone regeneration based on morphogenesis (Figure 36E) [535, 536]. Moreover, CoNFs are compatible with a series of biopolymers and inorganic materials, such as polyvinyl alcohol [537, 538], chitin/chitosan [522, 539, 540], silk fibroin [463], silver nanoparticles [541], minerals [542], ceramics [543], thus can be further functionalized into versatile composites to match the requirements of the applications in blood vessels, heart valves, nervous and bone tissue engineering.

5.4.1.2 Wound suture and healing materials: CoNFs are good candidates for developing mechanically robust and biodegradable materials for wound sutures and reconstructive surgery (Figure 37A) [544–546], due to their outstanding hemostatic and antibacterial properties, low antigenicity as well as the biocompatibility [545]. CoNFs have been formulated in diverse forms including powders, fibers, membranes, sponges and composites with other functional materials that act as wound dressing materials [547, 548] [555, 556]. These elements were applied for healing chronic wounds, ulcers treatment and particularly for skin replacement and covering burn wounds [549, 550]. Several advantages of the CoNF-based materials for wound sutures include reduced inflammation by covering the internal tissues of the burn wounds, absorption of wound secretions, and promotion of the growth of epithelial cells and thereby reducing contraction of the wounds [551].

Collagen- and CoNF-based materials were already afforded for human dermal substitutes in wound dressing applications (Figure 37B) [545, 552]. These products are conformable at room temperature for the various anatomic sites and the materials can also be stored for extended time frames. Wound dressing materials with designated functions, and surgical adhesives for preventing air leaks from damaged lungs [553] are examples of collagen products. In addition, biocompatible polymers (e.g., poly(vinyl alcohol) [553] and poly(glutamic acid) [554]), polysaccharides (e.g. alginate, cellulose, chitin/chitosan) [540, 555], proteins (e.g. sericin, silk protein) [556, 557], growth factors (e.g., platelet-derived growth factor, transforming growth factor, epidermal growth factor) [558] and metals (e.g. silica, silver) [559, 560] have also been integrated with CoNFs to produce optimized CoNF-based composite biomaterials (Figure 37C).

5.4.1.3 Drug delivery and disease diagnosis: In many drug delivery systems, CoNFs are the primary component, because the triple-helix structure in CoNFs can interact with a variety of molecules and further trigger biological events [561] [569]. CoNFs have been formulated into nano-/micro-particles, injectable dispersion/hydrogels, membranes, pellets, scaffolds and coatings[558]. These drug delivery systems are compatible with a wide range of therapeutic agents (Figure 37D) with length scales from molecule-size (chemicals, DNA, proteins) to micro-size (cells). The binding sites of CoNFs can be designed to establish or inhibit specific interactions for active compound targeting or anchoring. For example, CoNFs were functionalized by dimethyl maleic anhydride and citraconic anhydride to provide active sites for binding hydrophilic drugs[562]; D-glucose was modified with the CoNFs matrix that showed enhanced entrapment of *Calendula officinalis* [563]; recombinant CoNFs were designed to bind discoidin domain receptors for cancer therapy [564].

The morphology and stability of CoNF-based materials in the external environment is controllable, which directly regulates active compound diffusion and drug release behaviors. For example, tannic acid and lignin coated CoNFs hydrogels presented 9–12 times slower doxorubicin release compared to the bare collagen hydrogels [565], while iron oxide cross-linked CoNF scaffold presented paramagnetic behavior which triggered drug release with the stimulus of a magnetic field (Figure 37E) [566]. Furthermore, collagen levels and their morphology in living bodies were related to various diseases and thereby can serve as makers for disease diagnosis (e.g., diabetic nephropathy, epidermolysis bullosa acquisita [567], liver fibrosis [568], etc.). The change of type I collagen levels during the progression of fibrosis was applied for earlier diagnosis of chronic liver disease [569]. Polarized images of CoNFs in bio-tissues could help in the diagnosis of pathological changes (via statistical analysis). The quantification of collagen cross-linked metabolites in urine provided specific indexes for cartilage and bone breakdown [570].

5.4.2 Food and cosmetics applications—Collagen is not only a natural nutrient (a cholesterol-lowering food) [571, 572], but also exhibits efficient dermal matrix synthesis, and is useful for the treatment of idiopathic pulmonary fibrosis immunotherapy [573], arthritis and rheumatoid arthritis [574]. In the food industry, CoNFs usually serve as edible meat casings, binders, emulsifiers and extenders in sausages and hams, and are also used as additives to improve water holding capacity and tastes of meats [575]. CoNFs were usually blended with sodium alginate, starch, and sodium carboxymethylcellulose to generate thin films for food packing [576]. These packing films prevented water and air migration and ensured the food quality and retained biodegradability.

CoNFs are widely used in the cosmetic industry and provide a substrate for human dermal fibroblasts proliferation, and the stimulation of hyaluronic acid synthesis by fibroblasts [577]. CoNFs also offer antioxidant and reparative properties for aging skin [578]. Therefore, most commercial cosmetics targeted to improve skin use CoNF-based products [579, 580]. As an example, CoNFs were combined with essential plant oil extracted from *Eucalyptus globulus*, *Mentha piperita*, *Origanum onites* and *Thymus vulgaris* for the production of cosmetic antimicrobial emulsions [581].

5.4.3 Environmental applications—Hides and animal skins are by-products of the food industry but can be converted into valuable leather with tanners [582]. The tanning process involves pre-tanning, tanning, wet finishing, and finishing, thus turning the raw hides and animal skins into imputrescible materials [582]. Traditional leather processing usually produces substantial solid and liquid wastes. Fortunately, as mentioned in section 4.4.1, a series of top-down methods have been established to extract CoNFs from the tanning wastes for various applications such as fertilizer (Figure 37F) [583]. The reactions of CoNFs with tannins and aldehydes were studied in earlier research [584] and the results indicated that CoNFs could be used as powders for tanning, glues, dialysis membranes and adsorbent materials [585, 586].

Environmental pollution, and in particular heavy metal ion pollutants have severely affected human health, because of accumulation in living organisms [587]. However, as mentioned above, most ultrafiltration membranes and adsorbents cannot or only can partially remove heavy metal ions from contaminated solutions. In the past decades, CoNFs have been widely used for fabricating heavy metal ion adsorptive materials (Figure 37G) [588, 589]. Through chelation, the amino groups in collagen provide binding sites for a wide range of metal ions, including but not limited to Cu(II), Ni(II), Zn(II), Pt(II), Pd(II), Fe(III), Au(III), Pt(IV), V(V) and Cr(VI) [590–594]. A variety of CoNF materials, such as fibers, membranes, porous hydrogels and scaffolds, have been explored for the elimination of heavy metal ions from contaminated waters. These studies indicated that CoNF-based materials have remarkable adsorption selectivity and high efficiency for removal of heavy metal ions [591]. Furthermore, as for the CNFs, ChNFs and SNFs, CoNFs also can be integrated with other materials for water treatment. For example, CoNFs have processed into composite systems with Zr(IV) for fluoride adsorption [595], Fe(III) and Al(III) for phosphate removal [588] and tannins for uranium adsorption [596].

6. Conclusions and Outlook

A series of excellent reviews have summarized the structure, process, and applications of CNFs and CoNFs, but much less attention has been placed on ChNFs and SNFs. However, these four biopolymer nanofibrils are worth comparative studies, not only because they are four of the most abundant biopolymers on the planet, but also because they are four prototypes that represent how nature has used different elements to build materials with similar constructions [1]. For example, although CNF, ChNFs, and SNFs are composed of three distinct chemical compositions at the chemical unit level, they share several critical and general material constructions at the higher structural levels, i.e., the nanoconfinement of nanocrystal size, the orientation of nanofibrils and sophisticated hierarchical structures[1]. These common structural features enhance the physical (mechanical and optical) properties of the biological materials and also support biological functions, such as structural support, defense and prey capture [358, 597, 598]. Accordingly, investigation of these common material constructions in CNFs, ChNFs, SNFs, and CoNFs offers the opportunity to disclose universal material designs in nature and inspire the fabrication of biomimetic materials.

To obtain biopolymer nanofibrils, a series of top-down and bottom-up approaches have been developed. However, compared with CNFs, the method to extract ChNFs remains less

addressed. There are strong chemical and structural similarities with these two biopolymer nanofibrils, thus the methods used for isolation of CNFs, such as TEMPO-mediated oxidation, acid hydrolysis, etc., have been successfully utilized to extract ChNFs from exoskeletons of crustaceans. More recently, similar methods have also been used for obtaining SNFs and CoNFs. These results indicate the ways to produce each type of nanofibrils have potential to be transformed into another biopolymer nanofibril system. However, when using or creating a new nanofibril isolation route (top-down method) to generate biopolymer nanofibrils, a cradle-to-grave life cycle assessment (LCA) [328, 599, 600] is urgently needed to address the economic and scientific issues related to their production and utility, as well as their environmental impact. In particular, because most of the established methods are energy intensive and require a series of chemical reagents, these options can undermine the green and environmentally friendly features of biopolymer nanofibrils. Accordingly, the self-assembly of biopolymers (bottom-up method) were considered as a more sustainable method to produce biopolymer nanofibrils. However, unlike proteins (such as silk fibroin and collagen), the self-assembly processing of polysaccharides has been less investigated. Also, the mechanical and thermal properties of regenerated biopolymer nanofibrils are usually inferior to the natural nanobuilding blocks consisting of the same components.

From the perspective of biopolymer nanofibril applications, CNFs, ChNFs, SNFs and CoNFs have important common features from their nano-size effects, including ultra-high specific surface areas and length to diameter ratios [276–279], nanoporous structure [267, 280, 281], nanoconfinement effects [112, 114, 282] and optical transparency [267]. These unique characteristics shared by biopolymer nanofibrils offer the probabilities to design and apply these materials in similar routes. For instance, these materials have been successfully assembled into microspheres, fibers, nanopapers, membranes and various 3D materials (e.g., gels, foam, scaffolds, and 3D printed constructions) [1]. Among them, microspheres are good candidates as drug and cell carriers. The fibers are useful for producing mechanically enhanced materials. Membranes and nanopapers are widely used for flexible optical and electronic devices, water filtration, substrates for batteries as well as fracture-resistance materials. Gels, foams, scaffolds and 3D printed constructions have broad applications in tissue engineering, as inorganic templates, for water/oil separation, to support cells, and for mechanically enhanced bulk materials, among many other examples. However, the emerging applications of CNFs, e.g., photonic crystal devices, chiral catalysis, and carbonization, have been addressed to a lesser extent with ChNFs, SNFs, and CoNFs. Of note, all of these zero- to three- dimensional material formats can be fabricated from both isolated and self-assembled nanofibrils. However, for practical applications, the isolated nanofibrillar materials are more suitable for manufacturing mechanically enhanced materials, while materials produced by self-assembled nanofibrils are more suitable for biomedical applications due to their green and aqueous based processes. The integration of these biopolymer nanofibrils in a material system would be another interesting subject for future investigations, because these three materials not only have similarities but also are complementary in several physical aspects. For example, in biological materials, CNFs and ChNFs feature high strength while SNFs and CoNFs exhibit superior toughness. In engineering materials, top-down CNFs have advantageous mechanical performance but

present poor biocompatibility and biodegradability. In contrast, the materials generated from SNFs and CoNFs exhibited biocompatibility, but their mechanical properties still have room for improvement. Therefore, the combination of these biopolymer nanofibrils in materials can compensate for the limitations of individual components and have potential to enhance the performance and function of the final materials.

In summary, through this review, we established a global view to understanding the differences and similarities in structure, processing, and applications of four of the most abundant biopolymer nanofibrils. The fundamental understanding of these biological nanomaterials can accelerate the development of new methods to obtain new biopolymer nanofibrils and to produce novel nanomaterials, and also offer the new insights into the combination of different biopolymer nanofibrils in material systems for developing smart bionanomaterials.

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Figure 1. Hierarchical structures of CNFs, ChNFs, SNFs and CoNFs in wood, arthropod exoskeleton, spider silk and bone.

Cellulose

Chitin

Silk

Collagen

Figure 2.
Timeline of the structural characterization and preparation of biopolymer nanofibrils.

Figure 3.

The structure of the Type I collagen.

A, the chemical structure of glycine, proline and hydroxyproline. B, the structure of tropocollagen. C, AFM image of tropocollagen [58]. Copyright 2013. Reproduced with permission from Elsevier Ltd.

Figure 4.

Molecular deformation mechanisms of the wood cell wall.

A, The H-bonding network, indicated by the dash lines, in the crystalline cellulose [73].

Copyright 2008. Reproduced with permission from American Chemical Society. B, The atomistic structure of wood cell wall material, in which the crystalline cellulose phases are sandwiched with an amorphous matrix composed of hemicellulose and lignin. Shear loading on the model reveals two deformation mechanisms of the model: (M1) the reorganization of the molecules within the matrix and (M2) the sliding of matrix molecules along the cellulose surface with breaking and reconstruction of H-bonds [98]. Copyright 2015. Reproduced with permission from Elsevier Ltd.

Figure 5. Mechanical properties of chitin-protein interfaces. A, The crystal structure of chitin and the internal H-bonding network (red dotted lines). B, Atomistic models for studying the interaction between protein and chitin with *a*-helix and *b*-sheet being detached from the chitin surface [102]. Copyright 2013. Reproduced with permission from Springer.

Figure 6. Computational modeling of natural SNFs.

A, Fracture mechanism of small and large β -sheet nanocrystals during pull-out simulations. B, Schematic phase diagram to show the interplay of the parameters h and L (h is strand length and L is the nanocrystal size) in defining the properties of nanocrystals. The formation of confined β -sheet nanocrystals with critical strand length h^* and critical nanocrystal size L^* provides maximum strength, toughness, and stiffness. S =schematic plot of the strength of a β -strand as a function of strand length h , F =strength of nanocrystal as a function of crystal size L , T =toughness of nanocrystal as a function of crystal size L . C, The relationship between β -sheet nanocrystal dimension and mechanical properties. A-C reproduced with permission from [7]. Copyright 2010. Springer Nature. D, Force-displacement curves of the β -sheet nanocrystals with different dimensions under lateral loading. E, Stress-strain curves of spider silk based on different β -sheet nanocrystal sizes, ranging from 3 to 10 nm. D and E reproduced with permission from [13]. Copyright 2010. American Chemical Society. F, Finite element simulation of fibril interactions, showing

deformation and the maximum principal stress. Shear is applied from left to right on the lower fibril [123]. Copyright 2011. Reproduced with permission from American Chemical Society. G, Loading conditions used in the simulations to understand the impact of the defects on fiber mechanical properties, implementing tensile [mode I, (1) and (2)] and shear [mode II, (3) and (4)] loading with varied aspect ratios. H, Dependence of the failure strain and failure stress on the fibril size H, as well as a direct comparison with experimental results and the mechanical behavior of a defect-free silk fiber. G and H reproduced with permission from [112]. Copyright 2011. American Chemical Society.

Figure 7.

The structure and atomistic model of collagen.

A, The hierarchical structure of collagen fibers. Three polypeptide chains composed of amino acids win around each other to form tropocollagen at the nano-scale. Tropocollagens further assemble into fibrils in a staggered fashion and fibrils assemble collagen fibers [134]. Copyright 2006. Reprinted with permission from National Academy of Sciences. B, The full atomistic model of tropocollagen with various content of minerals [133]. Copyright 2011. Reprinted with permission from American Chemical Society.

Figure 8.

Preparation of CNFs through mechanical nanofibrillation methods.

A and B, Digital photograph and working mechanism of a high-pressure homogenizer. B reproduced with permission from [143]. Copyright 2011. John Wiley & Sons, Inc. C and D, TEM images of CNFs isolated from (C) bamboo and (D) rice straw by nanofibrillation of the purified cellulose pulps using a high-pressure homogenizer. C and D reproduced with permission from [144]. Copyright 2014. Elsevier Ltd. E and F, Digital photograph and working mechanism of a grinder. E and F reproduced with permission from [151]. Copyright 2013. Elsevier. G and H, FE-SEM images of wood CNFs isolated by nanofibrillation of the cellulose pulps using a grinder. G and H reproduced with permission from [152]. Copyright 2007. American Chemical Society. I, Digital photograph a high-speed blender. J, FE-SEM image of wood CNFs isolated by nanofibrillation of the cellulose pulps using a high-speed blender. I and J reproduced with permission from [153]. Copyright 2011. American Chemical Society. K, Digital photograph a high-intensity ultrasonicator. L, FE-SEM image of wood CNFs isolated by nanofibrillation of the cellulose pulps using a high-intensity ultrasonicator. L reproduced with permission from [160]. Copyright 2014. John Wiley & Sons, Inc.

Figure 9.

Preparation of CNFs through chemical modification combined with mechanical nanofibrillation methods.

A, Digital photograph of carboxymethylated CNF gel at 2 wt% in water. A reproduced with permission from [178]. Copyright 2013. Cambridge University Press. B, TEM image of wood carboxymethylated CNFs. The scale bar is 500 nm. B reproduced with permission from [176]. Copyright 2008. American Chemical Society. C-F, TEM images of CNFs disintegrated after TEMPO-mediated oxidation of never-dried samples: (C) bleached sulfite wood pulp, (B) cotton, (C) tunicin, and (D) BC. Inset in C is a transparent suspension of 0.1% CNFs of never-dried sulfite wood pulp TEMPO-oxidized with 2.5 mmol NaClO per gram of cellulose. C-F reproduced with permission from [181]. Copyright 2006. American Chemical Society.

Figure 10.

Preparation of CNCs through strong acid hydrolysis methods.

A-C, TEM images of CNCs obtained by H₂SO₄ hydrolysis of (A) cotton, (B) Avicel, and (C) tunicate cellulose. Insets: enlarged views of some individual particles. A-C reproduced with permission from [192]. Copyright 2008. American Chemical Society. D and E, TEM images of CNCs obtained by hydrochloric acid hydrolysis of (D) tunicate and (E) cotton. D reproduced with permission from [194]. Copyright 2007. American Chemical Society. E reproduced with permission from [160]. Copyright 2014. John Wiley & Sons, Inc. F, AFM image of CNCs obtained by phosphoric acid hydrolysis of cotton. F reproduced with permission from [195]. Copyright 2013. American Chemical Society.

Figure 11.

Preparation of BC through bottom-up methods.

A, Scheme for the formation of BC. A reproduced with permission from [208]. Copyright 1998. Elsevier. B, BC layers grown with different culture time (maximum 4 weeks). B reproduced with permission from [199]. Copyright 2000. Kluwer Academic Publishers. C-E, Synthesis of cellulose in *Acetobacter xylinum*. C-E reproduced with permission from [211]. Copyright 1976. National Academy of Sciences. F and G, FE-SEM images of BC pellicles: (F) shows the planar extensively cross-linked structure, whereas (G) shows the relatively weakly crosslinked layers in the thickness direction. F and G reproduced with permission from [212]. Copyright 2008. John Wiley & Sons, Inc.

Figure 12.
Overview of mechanical nanofibrillation methods for the preparation of ChNFs.

Figure 13.

Representative microscopy images of ChNFs prepared by mechanical nanofibrillation.

A, SEM images of α -ChNFs from crab shell [228]. Copyright 2009. Reproduced with permission from American Chemical Society. B, SEM images of α -ChNFs from mushroom [229]. Copyright 2011, Reproduced with permission from MDPI. C, TEM image of β -ChNFs from squid pen.

• **TEMPO-mediated oxidation**

Materials:

α -chitin from crab shells
 β -chitin from tubeworm

• **Anionically charged ChNFs:**

crab shell α -chitin nanowhiskers
 (width: ~ 8 nm, length: ~ 340 nm)
 tubeworm β -ChNFs
 (width: 20-50 nm, length: microns)

• **Partially (surface) deacetylation**

Materials:

α -chitin from crab shells

• **Cationically charged ChNFs:**

crab shell α -chitin chitin
 nanofibril/nanowhisker mixtures
 (width: ~ 6 nm, length: ~ 300 nm or
 microns)

Figure 14.

Elucidation of the mechanism and process of the preparation of ChNFs by TEMPO-mediated oxidation or partial deacetylation combined with mechanical treatment.

Figure 15.

Representative microscopy images of ChNFs prepared by chemical modification combined with mechanical treatment.

A, crab shell α -chitin nanowhiskers prepared by TEMPO-mediated oxidation [236].

Copyright 2008. Reproduced with permission from American Chemical Society. B, crab shell α -ChNFs and ChNCs mixtures prepared by partially deacetylation [239]. Copyright 2010. Reproduced with permission from Elsevier Ltd. C, crab shell α -ChNFs prepared by esterification followed by mechanical treatment [238]. Copyright 2016. Reproduced with permission from Elsevier Ltd. D, β -ChNFs prepared by TEMPO-mediated oxidation [237]. Copyright 2009, Reproduced with permission from Elsevier Ltd.

Figure 16.

A, TEM image of chitin nanowhiskers prepared by acid hydrolysis from shrimp shell [221]. Copyright 2007. Reproduced with permission from American Chemical Society. B, AFM image of chitin nanowhiskers prepared by acid hydrolysis from crab shell [251]. Copyright 2012. Reproduced with permission from Elsevier Ltd.

• *Liquid exfoliation process (Top-down approach)*

• *Self-assembly process (Bottom-up approach)*

Figure 17.

The methods for production of SNFs.

A and B, Schematic of the top-down approaches to produce SNFs [273]. Copyright 2016. Reproduced with permission from John Wiley & Sons Inc. C, Schematic of the bottom-up approaches to produce SNFs [276] (Copyright 2014. Reproduced with permission from Royal Society of Chemistry) and [281] (Copyright 2017. Reproduced with permission from American Association for the Advancement of Science).

Top-down methods

Bottom-up methods

Self-assembly on inorganic surface

Figure 18.

Structural features of the SNFs obtained from top-down methods, bottom-up methods and assembled on inorganic surface.

A, SEM image of SNF produced from ultrasound processing [159]. Copyright 2007.

Reproduced with permission from AIP Publishing LLC. B, SEM image of SNF produced from FA/CaCl₂ dissolution [275]. Copyright 2014. Reproduced with permission from The Royal Society of Chemistry.

C, SEM image of SNF produced from HFIP-based liquid exfoliation [273]. Copyright 2016. Reproduced with permission from John Wiley & Sons Inc. D, AFM image of SNFs obtained from tuning solvent systems [274]. Copyright 2016. Reproduced with permission from John Wiley & Sons Inc.

E, AFM image of SNFs produced from 7 vol% EtOH induced self-assembly of silk fibroin [267]. Copyright 2014. Reproduced with permission from John Wiley & Sons Inc.

F, AFM image of SNFs produced from heating induced self-assembly of silk fibroin [281]. Copyright 2017. Reproduced with permission from American Association for the Advancement of Science.

G, silk fibroin self-assembly on the reduced GO surface [299]. Copyright 2014. Reproduced with permission

from American Chemical Society. H, Top, the self-assembly of silk fibroin on the reduced graphene, GO and silicon dioxide surfaces. Bottom, the simulation of the interaction between silk fibroin and inorganic surfaces [298]. Copyright 2016. Reproduced with permission from John Wiley & Sons Inc.

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Figure 19.

A typical route for extracting collagen from the calcified tissue. The flow chart was redrawn according to reference [308].

Figure 20.

Development of CNFs and CNCs as nanobuilding blocks for advanced composite applications.

A-D, Composite preparation by a CNC template approach. A, Schematic of the template approach to well-dispersed CNC/polymer composites. i, A non-solvent is added to a CNC dispersion in the absence of any polymer. ii, Solvent exchange promotes the self-assembly of a CNC gel. iii, The gelled CNC scaffold is imbibed with a polymer by immersion in a polymer solution, before the nanocomposite is dried (iv) and compacted (v). B, Digital photograph of a CNC aerogel, prepared by supercritical extraction of a CNC acetone gel (A, ii) with a CNC density of 15 mg ml^{-1} . C, Same object as in B, imaged through crossed polarizers. D, SEM image of the same material (scale bar is 200 nm). A-D reproduced with permission from [340]. Copyright 2007. Springer Nature. E-G, Stimuli-responsive polymer composites inspired by the sea cucumber dermis. E and F, Digital photograph of sea cucumber in relaxed (E) and stiffened (F) state demonstrating the firming of dermal tissue in the vicinity of the contacted area. G, Schematic representation of the architecture and switching mechanism in the artificial composites with dynamic mechanical properties. In the “on” state, strong hydrogen bonds between rigid, percolating CNCs maximize stress transfer and in addition to that the overall modulus of the composite. The interactions are switched “off” by the introduction of a chemical regulator that allows for competitive hydrogen bonding. E-G reproduced with permission from [341]. Copyright 2008. American Association for the Advancement of Science. H-J, Fabrication of flexible magnetic aerogels using CNFs as templates. H, Schematic showing the synthesis of flexible magnetic aerogels. I, SEM image of a 98% porous magnetic aerogel containing cobalt ferrite nanoparticles after

freeze-drying. Right inset: nanoparticles surrounding the CNFs. Left insets: photograph and schematic of the aerogel. J, HRTEM image of a single particle from magnetic aerogels showing the lattice fringes corresponding to the (111) reflections of the spinel structure, and the corresponding distance. H-J reproduced with permission from [348]. Copyright 2010. Springer Nature. K-O, Fabrication of thermally insulating and fire-retardant anisotropic foams based on CNFs and GO. K, Schematic illustration of the freeze-casting process, highlighting the growth of anisotropic ice crystals surrounded by walls of the dispersed nanoparticles. L, Digital photograph of the nanocomposite foam. M, SEM cross-section image of a freeze-cast nanocomposite foam. N, Three-dimensional reconstruction of the tubular pore structure of the nanocomposite foam derived from X-ray microtomography. O, X-ray microtomography image showing that the tubular pores are straight and several millimeters long in nanocomposite foam. K-O reproduced with permission from [349]. Copyright 2015. Springer Nature.

Figure 21.

Development of CNFs for optical applications.

A, Digital photograph of a transparent and flexible BC reinforced polymer composites. A reproduced with permission from [206]. Copyright 2005. John Wiley & Sons, Inc. B, Digital photograph of a transparent and flexible wood CNF nanopaper. B reproduced with permission from [353]. Copyright 2009. John Wiley & Sons, Inc. C, Luminescence of an organic light-emitting diode deposited onto a transparent BC nanocomposite. C reproduced with permission from [212]. Copyright 2008. John Wiley & Sons, Inc. D, Luminescence of an organic light-emitting diode deposited onto a flexible and transparent wood CNF nanocomposite. D reproduced with permission from [351]. Copyright 2009. Elsevier Ltd. E, Schematic of the chiral nematic ordering present in H_2SO_4 -hydrolyzed CNCs, along with an illustration of the half-helical pitch $P/2$ (~ 150 – 650 nm). F, POM image of the mesoporous silica film obtained from the calcination of the H_2SO_4 -hydrolyzed CNCs/silica composite film. G, Photograph showing the different colours of mesoporous silica films obtained from the calcination of various composite films. H, Transmission spectra of the mesoporous silica films. I, Synthetic route to mesoporous photonic cellulose. E-H reproduced with permission from [360]. Copyright 2010. Springer Nature. J, Photographs and K, UV/Vis (solid lines) and CD spectra (dashed lines) of mesoporous photonic cellulose soaked in EtOH/ H_2O at different ratios as indicated. I-K reproduced with permission from [368]. Copyright 2014. John Wiley & Sons, Inc.

Figure 22.

Development of CNF-derived nanopapers for electronic applications.

A, Digital photographs of original nanopaper (left), CNT@nanopaper (middle) and silver nanowire@nanopaper. B-D, Flexible performance of the transparent conductive nanopapers. (B) Resistance values (184Ω) of silver nanowire@nanopaper after mountain folding. (C) The lighting of a green LED placed between mountain- and valley-folded silver nanowire@nanopapers. (D) Paper craft by using transparent conductive papers. A-D reproduced with permission from [372]. Copyright 2014. Springer Nature. E, Schematic illustrations of the fabrication processes for stretchable CNF/graphene nanopapers. F, Example images of the free-standing flexible nanopaper and stretchable nanopaper. G-I, Example images of the CNF/graphene nanopaper sensors stretched in the X-, Y- and Z directions. J, Corresponding response curves for stretching in three directions. E-J reproduced with permission from [373]. Copyright 2014. John Wiley & Sons, Inc.

Figure 23.

Development of CNF-derived aerogels for electronic applications.

A, Fabrication of CNF/CNT conductive aerogels. A reproduced with permission from [376].

Copyright 2013. John Wiley & Sons, Inc. B, SEM image of BC aerogel. C, SEM image of BC-derived carbon aerogel.

D and E, The pressure response of BC-derived carbon aerogels.

D, Plot of the electric resistance variation with compressive strain. The inset in (D) shows an in-situ measurement of the electric resistance during the compression process. E, The variation of electric current with cyclic compression in a closed circuit. B-E reproduced with permission from [379]. Copyright 2013. John Wiley & Sons, Inc. F and G, Resistance change of the BC-derived aerogel/PDMS composite under mechanical deformations.

F, Variation of the normalized resistance (R/R_0) of the composite as a function of tensile strain up to 80% in the first two stretch-release cycles. The inset shows the stretching process. (G) R/R_0 of the composite at a bend radius of up to 1.0 mm in the first bending

cycle. The inset shows the bending process. F and G reproduced with permission from [378]. Copyright 2012. Springer Nature.

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Figure 24.

Development of CNF-derived composite papers for energy storage applications.

A, Schematic representation of the overall fabrication procedure for unitized SEA. B, Schematic illustration of an *h*-nanomat cell with the unitized SEA configuration. A and B reproduced with permission from [392]. Copyright 2014. American Chemical Society. C, Schematic illustration depicting CNF/CNT-intermingled heteronet architecture of CM electrodes. D, Digital photographs showing the electrochemical activity of paper crane hetero-nanonet cell (I) and paper crane HN cell (II) (a left-bottom inset image). The left-top image is a digital photograph showing the operation of a mini toy car installed with the single-unit hetero-nanonet paper cell. C and D reproduced with permission from [393]. Copyright 2015. John Wiley & Sons, Inc.

Figure 25.

Development of CNF-derived carbon aerogels for energy storage applications.

A-F, BC-derived carbon aerogels for supercapacitors. A and B, STEM images of the carbon aerogels@MnO₂ with corresponding elemental mapping images of (A) C and (B) Mn. C and D, EFTEM images of the nitrogen-doped carbon aerogels with corresponding elemental mapping images of (C) C and (D) N. E, Scheme of the asymmetric supercapacitor device. F, Ragone plots of the supercapacitors. A-F reproduced with permission from [400]. Copyright 2013. John Wiley & Sons, Inc. G-I, BC-derived carbon aerogels for lithium-ion batteries. G, TEM images of the BC-derived carbon aerogels. H, TEM image of the carbon aerogel/SnO₂ composites. I, Comparison of cycling performances of carbon aerogel/SnO₂ composites and aggregated SnO₂ nanoparticles over 100 cycles at 100 mA g⁻¹. G-I reproduced with permission from [401]. Copyright 2013. John Wiley & Sons, Inc.

Figure 26.

Development of CNF/CNC-derived materials for energy conversion applications.

A, An organic solar cell on conductive CNF nanopaper. A reproduced with permission from [409]. Copyright 2013. Royal Society of Chemistry. B, Solar cell based on foldable and transparent conductive CNF nanopaper. B reproduced with permission from [410]. Copyright 2015. Springer Nature.

C, Device structure of solar cells on H_2SO_4 hydrolysis CNC substrates: CNC/Ag/PEIE/PBDTTT-C:PCBM/ MoO_3 /Ag. D, J - V characteristics of the solar cell on H_2SO_4 hydrolysis CNC substrate in the dark (thin black line) and under 95 mW/cm^2 of AM1.5 illumination (thick red line). C and D reproduced with permission from [411]. Copyright 2013. Springer Nature.

Figure 27.

Development of CNFs for environmental applications.

A-E, CNF-derived nanopaper filters for filtration. A, Schematic of the size-exclusion CNF nanopaper filter for nanoparticle removal. A reproduced with permission from [412].

Copyright 2014. John Wiley & Sons, Inc. B, SEM image of removed 20 nm gold nanoparticles using the CNF nanopaper filter. B reproduced with permission from [415].

Copyright 2016. Royal Society of Chemistry. C, AFM and TEM of CNF/silk fibroin nanostructure. z-scale: 10 nm. D, The model of periodically assembled silk backbones on

CNF surface. E, The as-prepared 200 nm nanopaper supported on PC filter before and after filtrating R₆G. C-E reproduced with permission from [417]. Copyright 2017. American Chemical Society. F, G, CNF-derived carbon aerogels for oil/water separation. F, Digital photograph showing the absorption of gasoline by a piece of BC-derived carbon aerogel from the water. F reproduced with permission from [379]. Copyright 2013. John Wiley & Sons, Inc. G, Digital photograph showing the absorption of chloroform by a piece of wood CNF-derived carbon aerogel from the water. G reproduced with permission from [394]. Copyright 2016. John Wiley & Sons, Inc.

Figure 29.

Optical and electronic applications of ChNF-based materials.

A, Optically transparent nanocomposites using fibrillated ChNFs with three different types of acrylic resins [230]. Copyright 2012. Reproduced with permission from Hindawi. B, Photograph (with AFM image inset) of a chitin reflective optical grating replica on a silicon substrate, molded from a grating with 1200 grooves/mm[264]. Copyright 2011. Reproduced with permission from John Wiley & Sons Inc. C, Fabrication approach of the N-doped ChNF aerogel from prawn shells[455]. Copyright 2015. Reproduced with permission from Elsevier Ltd. D, All-chitin derived flexible electric circuit with the thickness of carbon layer approximately 1 μm [456]. Copyright 2017. Reproduced with permission from John Wiley & Sons Inc. E, Organic light-emitting diodes device on ChNF paper to fabricate substrates for flexible green electronics [452]. Copyright 2016. Reproduced with permission from John Wiley & Sons Inc.

Figure 30.

Environmental applications of ChNF-based materials.

A, Observation of ChNCs with stabilizing properties in oil/water emulsions after 24 h of storage at room temperature [462]. Copyright 2011. Reproduced with permission from Elsevier Ltd. B-C, Hydrogels via gas phase coagulation (top) indicated B) adsorption of Reactive Blue 19 (bottom) owing to negatively charged ChNFs and C) adsorption of Basic Green 4 (bottom) owing to positively charged ChNFs [241]. Copyright 2016. Reproduced with permission from American Chemical Society.

Figure 31.

Regulation of silk fibril alignments in one to three dimensions.

A, Schematic of dry-spinning of silk microfibril/HFIP dope. B and C, the polarized optical (B) and SEM image (C) of regenerated silk fibers produced from silk microfibril spinning dope. D, SEM image of a yarn-like spiral regenerated silk fiber produced by rotating the collector in a plane direction that perpendicular to the fiber axis. E, Three-dimensional cell patterns generated on yarn-like spiral and as-spun regenerated silk fibers. Fluorescent images show the preferential alignment of HDFs (green) along the axes of the yarn-like spiral and as-spun regenerated silk fibers (red). A-E reproduced with permission from [115]. Copyright 2017. Springer Nature. F, Schematic and visual images of hydrogel-forming process in the electric field. G, polarized optical images of SNF hydrogels before electric field treatment (left) and after treatment (right). H, High-resolution SEM image of the nanostructure on the layers. The layers are composed of aligned SNFs. I, Alignment of stem cells on the surface of the electronically oriented silk hydrogels for 3 days incubation. F-I reproduced with permission from [470]. Copyright 2016. John Wiley & Sons Inc. J, Birefringence and corresponding internal nanofibrillar morphology of a ring-anchored fiber.

Increasing stress and birefringence correspond to increasing nanofibrillar alignment and orientation. K, buckling of high-aspect-ratio beams with increasing birefringence/alignment of fibers induced via contraction. L-M, Visual image of a web taken between two polarizer films. This six-anchor nanofibrillar web (~2.5 mg) supporting an 11 g point load. J-N reproduced with permission from [296]. Copyright 2017. Springer Nature.

Figure 32.

Injectable SNF hydrogels.

A, Thixotropic SNF-based hydrogel obtained by centrifugation of SNF dispersion [276].

Copyright 2014. Reproduced with permission from The Royal Society of Chemistry. B, Injectable and pH-responsive SNF hydrogels generated from heating induced self-assembly and their applications in sustained anticancer drug delivery [279]. Copyright 2016.

Reproduced with permission from American Chemical Society. C, SNF/laponite nanoplatelets injectable hydrogels produced from sonication processing with enhancing

mechanical performance and bioactivity [477]. Copyright 2016. Reproduced with permission from American Chemical Society.

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Figure 33.

SNF assisted synthesis of the functional inorganic nanomaterials.

A, SEM image of gold nanoplatelets synthesized from liquid exfoliated SNF solution [273].

Copyright 2016. Reproduced with permission from Wiley & Sons Inc. B, The gold nanocrystals produced from EtOH-induced SNF solution [487]. Copyright 2014.

Reproduced with permission from Springer. C, the oriented growth of gold nanoparticles following SNFs. D, One-step synthesis of Fe_3O_4 -silk composite nanoparticles. E, SNF-modulated morphology control of CaCO_3 nanocrystals using silk nanofibrils with the length of about 50 nm. C-E, reproduced with permission from [272]. Copyright 2014. Reproduced with permission from American Chemical Society.

F-H, SNF/HAP nanocomposites. G and H indicate that HAP nanocrystals can homogeneously disperse on the SNF network without aggregates. F reproduced with permission from [488]. Copyright 2016. The Royal Society of Chemistry. G and H reproduced with permission from [281]. Copyright 2017. American Association for the Advancement of Science.

Figure 34.

SNF based nanoporous membranes.

A, Schematic of the vacuum filtration devices. B, Visual image of the SNF membranes produced from liquid exfoliated SNF dispersion. C, and D, A cross-sectional SEM image of the liquid exfoliated SNF membrane under low and high magnification. E and F, Visual appearance of the membrane with 5:5 SNFs: amyloid fibrils under visual (E) and cross-polarized light (F) observation. G, A cross-sectional SEM image of the membrane with 100% SNFs. H, Quantum dot patterned SNF membranes under UV light. I) Visual image of gold single crystal nanoplatelets (92 wt%) patterned SNF membranes. J, Visual images show the attached SNF based flexible electronic devices deformed with the deformation of pig ear. K, SNF/ Kevlar nanofibril based electronic devices [489]. Copyright 2017. Reproduced with permission from American Chemical Society. L, Magnetic functionalization and tensile properties of the membrane (SNFs:amyloid fibrils:magnetic nanoparticles weight ratio of

70:10:20), as prepared by vacuum filtration. M, Shape-memory properties of the magnetic composite membrane when exposed to the combined presence of an external magnetic field and water. B-D and H-J reproduced with permission from [273]. Copyright 2016. John Wiley & Sons Inc. E-G, L and M reproduced with permission from [267]. Copyright 2014. John Wiley & Sons Inc.

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Figure 35.

SNF based filtration membrane for water purification.

A, pristine SNF ultrathin filtration membrane. B, Schematic of the rejection process of SNF membrane during the filtration. A and B, reproduced with permission from [280]. Copyright 2016. Reproduced with permission from American Chemical Society. C, SNF/amyloid nanofibril nanoporous membrane with tunable pore size [267]. Copyright 2014. Reproduced with permission from John Wiley & Sons Inc. D, Coarse-grained computational MD simulations for SNF/HAP assembly and deposition. E, the route to fabricate the SNF/HAP

membranes, and visualization of typical multilayer structures formed. D and E reproduced with permission from [281]. Copyright 2017. Reproduced with permission from American Association for the Advancement of Science.

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Figure 36.

Structure of CoNF-based materials.

A, CoNFs based multi-layered scaffold was fabricated to mimic the composition and microstructural properties of the superficial, intermediate and deep layers of the osteochondral region [526]. Copyright 2016. Reproduced with permission from Elsevier Ltd.

B, Hierarchical structure of the tendon [16]. Copyright 2007. Reproduced with permission from Nature Springer. C, Anisotropic alignment of CoNF laminar in cornea [530]. Copyright 2003. Reproduced with permission from Association for Research in Vision and Ophthalmology.

D, 2D (a,b,c,d) and 3D (e,f,g,h) reconstructions of CoNFs nano-sized bioactive glass and CoNFs hybridizing rolls with MicroCT images. More homogenous mineralization (i.e., greater carbonated hydroxylapatite formation) was achieved in red phase of these rolls with longer conditioning time in simulated body fluid [535]. Copyright 2011. Reproduced with permission from Elsevier Ltd. E, Fabrication of the hierarchical structure of hydroxyapatite (HA)-collagen composite. The self-assembling of composite materials was indicated with (I) CoNFs, (II) the HA crystals grow on the surface of organized CoNFs, and (III) paralleled organization of the mineralized CoNFs [536]. Copyright 2003.

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Figure 37.

Applications of CoNF-based materials.

A, CoNFs glue applied in lung surgery to reduce air leaks from the lung. (i) Pneumothorax in a treated rabbit at day 4, the lung border is marked by arrows. (ii) The adhesion is showing a giant-cell granuloma containing CoNFs glue debris [544]. Copyright 2000.

Reproduced with permission from Oxford University Press. B, Combined AlloDerm® and thin skin grafting for the treatment of (i) a scar contracture area on the anteromedial aspect of the left elbow, and (ii) postoperatively indicated the even releasing surface and adequate skin color of the elbow [545, 552]. Copyright 2014. Reproduced with permission from John Wiley & Sons, Inc.

C, Strategy of dye and growth factors anchoring in CoNFs to enable wound assessment as well as to expedite wound healing [545]. Copyright 2014. Reproduced with permission from John Wiley & Sons, Inc. D, Schematic of representative examples of various classes of molecules that interact with CoNFs, along with the representation of the interaction network of the three major CoNFs (types I, II and III) [558]. Copyright 2015.

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Reproduced with permission from American Chemical Society. F, Bandaging films (i) prepared with CoNFs recycled from the leather industry wastes, and (ii) application to skin [561]. Copyright 2016. Reproduced with permission from Royal Society of Chemistry. G, The SEM images of (i) CoNFs based hydrogels for metal ions adsorption. (ii) The smooth morphology of the metal-adsorbed surface is related to filling the surface pores by metal particles [588]. Copyright 2009. Reproduced with permission from Elsevier Ltd.