Fiber-Based Biopolymer Processing as a Route toward Sustainability

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Some of the most abundant biomass on earth is sequestered in fibrous biopolymers like cellulose, chitin, and silk. These types of natural materials offer unique and striking mechanical and functional features that have driven strong interest in their utility for a range of applications, while also matching environmental sustainability needs. However, these material systems are challenging to process in cost-competitive ways to compete with synthetic plastics due to the limited options for thermal processing. This results in the dominance of solution-based processing for fibrous biopolymers, which presents challenges for scaling, cost, and consistency in outcomes. However, new opportunities to utilize thermal processing with these types of biopolymers, as well as fibrillation approaches, can drive renewed opportunities to bridge this gap between synthetic plastic processing and fibrous biopolymers, while also holding sustainability goals as critical to long-term successful outcomes.

1. Introduction

Currently, over 350 million tons of plastics are produced every year, requiring ≈4–8% of the fossil fuel resources.[1] The plastics sector is estimated to account for 20% of total oil consumption by 2050.[2] This growing demand for plastics, along with the rising cost of raw materials, leads to a long-term challenge to meet the demands for plastics. Moreover, plastics generate significant environmental issues due to waste mismanagement and inefficient recycling systems; currently ≈80% of all marine debris contains plastics.[3] Predictions are that 600 million tons of plastic waste will end up in oceans by 2040 if no new strategies are implemented.[4]

The current lifecycle for synthetic plastics follows fossil fuel as resources, the conversion into the plastics, manufacture of products, and disposal into landfills and oceans (Figure 1a). This linear lifecycle for plastics leads to plastic pollution, economic losses, and a reduction in natural fossil resources. With the current recycling infrastructure, only 14% of current plastic-packaging waste is collected, and even with this recycling, 4% is lost during the process and 8% is redirected for the fabrication of lower-value applications.[5]

The long degradation half-lives of synthetic plastics, which break down in the environment over decades or centuries, is the central problem of sustainability.[6,7] Therefore, seeking sustainable and biodegradable alternatives to synthetic plastics have become a pressing task. Developing biodegradable products from renewable resources (e.g., natural biopolymers) represents the best option by fitting material design, production, use, and disposal into a circular materials lifecycle approach (Figure 1b).[8] Instead of losing fossil fuel resources into landfills and causing environmental burdens, natural biopolymers offer options for composting/degradation, thus, recycling the carbon back to environmental cycles for the regeneration of feedstocks. Besides being sustainable and biodegradable, fibrous structural biopolymers from plants (e.g., cellulose) and animals (e.g., silk, chitin) have unique hierarchical structures, with structural integrity, flexibility, and toughness. Therefore, fibrous structural biopolymers represent a class of material that could help to supplement or replace some conventional synthetic plastics. Additionally, the inherent biocompatibility of most natural biopolymers makes them important candidates for added-value applications, such as in regenerative medicine, drug delivery, and tissue-implantable devices. Among these natural biopolymers, cellulose, chitin, and silk fibroin represent the most abundant and investigated biopolymers in the categories of polysaccharides and proteins (Table 1).

The first step in this progression toward sustainability is to process fibrous biopolymers into material formats suitable for targeted applications. The sophisticated hierarchical structure of fibrous biopolymers, which consists of well-organized structural features from molecular to nano- to macroscopic length scales, employ extensive hydrogen bonding networks embedded within semicrystalline structures across all structural levels. This structural stability endows fibrous biopolymers with exceptional mechanical properties, but also generates challenges with the direct processing of the fibrous materials into useful material formats via traditional thermal processing methods that are widely used for synthetic polymer processing.
due to the low cost and relatively simple approaches. Alternatively, solution-based methods,\textsuperscript{[15–18]} where biopolymers dissolve at the molecular level, have traditionally been used for fibrous biopolymer processing to overcome or embrace the inherent structural stability of these native materials. The unique hierarchical organization of their fibrous structures prompts the use of fibrillation-based methods more recently developed for processing,\textsuperscript{[19,20]} where the native building blocks of the fibrous biopolymers are retained during processing. This gap between synthetic plastic processing (thermal) and fibrous biopolymer (solution and fibrillation based) processing approaches invariably results in major gaps in process utility, complexity, costs, and consistency in polymer outcome; thermoplastics are preferred. Fibrous biopolymers (e.g., cellulose, chitin, silk), by contrast, spur innovation and interest due to their remarkable structural features (hierarchy) and functional outcomes (e.g., mechanics), along with their green origins. To bridge this chasm and improve the utility of fibrous biopolymers, inroads to match plastics processing methods are needed. Thus, new approaches to move fibrous biopolymer processing into synthetic plastics processing streams are needed. By doing so, new utility will be realized, and in turn, improved polymer sustainability will be the outcome; utilizing naturally abundant fibrous biopolymer material feedstocks but with traditional synthetic

Table 1. Physicochemical and thermal properties of cellulose, silk fibroin, and chitin ($X_c$: degree of crystallinity; $T_g$: glass transition temperature; $T_m$: melting temperature; $T_d$: decomposition temperature; $\sigma$: tensile strength; $E$: Young’s modulus; $\varepsilon$: elongation at break, $U_t$: toughness).

<table>
<thead>
<tr>
<th>Materials</th>
<th>$X_c$ [%]</th>
<th>$T_g$ [°C]</th>
<th>$T_m$ [°C]</th>
<th>$T_d$ [°C]</th>
<th>$\sigma$ [GPa]</th>
<th>$E$ [GPa]</th>
<th>$\varepsilon$ [%]</th>
<th>$U_t$ [MJ m$^{-3}$]</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Cellulose (43–88)</td>
<td>200–250</td>
<td>260–270</td>
<td>260</td>
<td>0.1–2</td>
<td>0.7–17</td>
<td>0.5–0.7</td>
<td>58–270</td>
<td>58–270</td>
<td>0.2–27</td>
</tr>
<tr>
<td>Silk fibroin (30–34)</td>
<td>200–220</td>
<td>257–337</td>
<td>250–260</td>
<td>0.5–0.7</td>
<td>5–23</td>
<td>15–35</td>
<td>70–150</td>
<td>70–150</td>
<td>0.2–27</td>
</tr>
<tr>
<td>a) Chitin (57–83)</td>
<td>170–236</td>
<td>N/A</td>
<td>230–330</td>
<td>0.1–1.3</td>
<td>2.5–10</td>
<td>2.5–10</td>
<td>40–60</td>
<td>40–60</td>
<td>0.3–21</td>
</tr>
</tbody>
</table>

\textsuperscript{a)Values measured from materials from different sources; b)Values measured from bulk materials; c)Theoretical values; d)Values measured from the crystalline region of the materials or highly crystalline materials.}
plastics processing methods. The opportunity to start bridging this gap is upon us; here we review this progress and future vision. Recently, a heat compression-based method was developed to transform silk directly into compact bulk materials through molecular self-assembly in the solid state without added solvent, representing a major step toward sustainable, large-scale production of fibrous fiber-based products.\textsuperscript{[21,22]}

The similarities in structural hierarchy and thermal stability between various fibrous biopolymers (silk, cellulose, chitin) suggests a path forward with most of these systems. In this review, we provide a summary of biopolymer processing toward sustainability. We first highlight the unique chemical and hierarchical structures of natural biopolymer fibers and discuss common themes in biopolymer processing to highlight the differences with processing methods used with synthetic plastics. Using silk and cellulose as two exemplary fibrous biopolymers generated in high volume today, we then review the solution-based processing into materials of versatile morphologies and functions, with a focus on sustainability. Next, we introduce fibrillation methods and solid-based biopolymer thermal processing as new directions toward addressing both improved plastics-like outcomes and sustainability with fibrous biopolymer feedstocks. Canonically, fibrous biopolymers have not been compatible with thermal processing approaches, and fibrillation approaches were less studied. These new approaches provide a path forward for processing a broader range of fibrous biopolymer systems. Lastly, we discuss challenges as well as future prospects for fibrous biopolymer processing toward broader utility, with sustainability goals in mind.

2. Structure-Thermal Properties–Processing Relationships

A significant obstacle to substituting synthetic plastics with biopolymers is production cost. Current biopolymer processing methods are expensive when compared with conventional thermal-based plastic processing methods. The vast majority of synthetic plastics (\(\approx92\%\)) are thermoplastic, which can be melt and reshaped many times.\textsuperscript{[23]} The most abundant natural biopolymers generally do not have thermoplastic properties and are stabilized by extensive hydrogen bonding.

Structurally, thermoplastic polymers consist of repeat units or monomers, forming linear or lightly branched structures (Figure 2a). As the basic building blocks of thermoplastics, the linear or branched polymer chains are intertwined through weak forces and simple physical entanglements. Thermoplastic polymers can be amorphous or semicrystalline depending on crystalline regions. Amorphous polymers contain chain packing defects and polymer chains are typically held together by weak van der Waals or hydrogen bonding. Since mobility is not severely restricted, polymer chains can slip by each another at relatively low temperatures to break the weak interactions and reform into new ones. Semicrystalline polymers contain crystalline regions with arranged polymer chains in specific spatial and packing patterns, where the forces between polymer chains are mainly van der Waals or dispersion forces.\textsuperscript{[24]}

Owing to the relatively simple and flexible chain structure and dynamics, a typical semicrystalline thermoplastic undergoes a series of transitions when heated (Figure 3a). At relatively low temperature (below \(T_g\)), the weak interactions between the polymer chains in amorphous regions are disrupted and the amorphous structures are mobile and above the \(T_g\) become rubbery. Thermoplastic polymers usually exhibit \(T_g\)s between \(-103\) and \(227\,{}^\circ\text{C}\).\textsuperscript{[25]} When the temperature is increased further, the forces governing interactions between the polymer chains in the crystalline regions are disrupted and these regions melt \(\left(T_m\right)\). Usually, the \(T_m\) for semicrystalline polymers is \(1.25\) and \(2\) times of \(T_g\) (in Kelvin).\textsuperscript{[26]} The polymer chains thermally decompose at even higher temperature \(T_d\). When sufficient crystallizable amorphous structures exist, the polymer chains can reorganize and form new crystalline structures at crystallization temperature \(T_C\) (Figure 3a). Since the \(T_g\) and \(T_m\) are normally below and well separated from \(T_d\), polymers can be reshaped at elevated temperature, thus, thermoplastic behavior. The wide processing window for thermoplastics (Figure 3a) enables versatility in methods to achieve inexpensive and customizable mass production of plastic parts, including via compression molding, injection molding, extrusion, and 3D printing (Figure 3b).

Fibrous biopolymers resemble semicrystalline synthetic materials with both amorphous and crystalline regions, but are usually not thermoplastic. Compared to the simple linear or lightly branched structures of synthetic thermoplastic polymers, fibrous biopolymers often possess well-organized hierarchical structures dominated by a high density of hydrogen bonding. The structures of cellulose, silk fibroin, and chitin fibers (Figure 2b–d), although composed of different biomolecules at the molecular level, share common features at higher structural order initiated and controlled at the molecular level via the chemistry and interchain bonding. At the nanoscale, the biomolecules assemble into nanofibers with highly orientated nanocrystals embedded in less structured amorphous matrices. The nanofibers are further bundled into microfibers and then assembled into macroscopic structures or fibers, all of which is driven by the chemistry, monomer sequences and linkages between monomers (e.g., chirality, regioselectivity, etc.) encoded within the primary chains for each polymer.

When biopolymers are subject to heating, instead of undergoing distinct \(T_m\)s and/or \(T_d\)s, they usually decompose at high temperature without these phase transitions (Figure 3c, Table 1). Fibrous biopolymers often have a high \(T_g\) and \(T_m\) due to their complex, extensively bonded structures. For example, some polysaccharide fibrils, e.g., cellulose and chitin, possess crystalline regions containing parallel stacks of chains with a high density of intermolecular hydrogen bonds between hydroxyl groups and oxygens of adjacent molecules.\textsuperscript{[27]} Generally, the hydrogen bond is 5 to 30 kJ mol\(^{-1}\) stronger than van der Waals interactions,\textsuperscript{[28]} thus, requiring higher temperature to break these bonds and mobilize the chains.\textsuperscript{[29]} Therefore, such dense hydrogen-bonded networks result in undetectable \(T_m\)s for cellulose and chitin before decomposing at 260 °C\textsuperscript{[30,31]} and 230–330 °C,\textsuperscript{[14]} respectively. Domains in the amorphous regions are also arranged with some order, connecting the crystalline regions with relatively low free volume. Together with the impact of the large crystalline regions, these chains have low mobility and result in a high \(T_d\) at 200–250 °C for cellulose\textsuperscript{[32]} and 170–236 °C for chitin.\textsuperscript{[14]} Additionally, plasticizers
are difficult to apply to the fibers directly because of the compact structures that prevent diffusion inside the hierarchical structures, even to the exclusion of water.

Similarly, the β-crystallites in silk fibers, composed of several adjacent β-sheets from different molecules, resemble the crystalline regions in synthetic thermoplastic polymers. The main
forces that maintain the structure of β-crystallites by linking different β-sheets are the hydrophobic interactions/van der Waals interactions.[34] Both degummed silk fibers and regenerated silk films have a $T_d \approx 250–260$ °C,[35–37] directly related to the fission of covalent bonds in the protein chain backbone (primary structure).[38] At such temperatures, silk degrades before melting. Native silk fibers have a high $T_g$ of 200–220 °C due to its high β-sheets crystallinity.[39,40] At such temperatures, although the amorphous regions of silk fiber become mobile, silk molecules did not gain sufficient chain mobility to allow thermal processing. In contrast to previous thinking that silk would not melt without degradation, a canonical view since Pauling’s earliest report of the β-sheet structure in silk fibers,[45] the melting of silk crystalline structures was first reported using fast scanning calorimetry at 2000 K s$^{-1}$.[13] Here, $T_m$s of 257 and 337 °C were observed for regenerated silk films and degummed silk fibers, respectively.[13] However, the extremely high heating rate as well as the small sample size challenged scale up of the process for bulk silk materials.

Due to the difficulties in processing the most prominent native fibrous biopolymers (e.g., cellulose, silk, chitin) using thermal methods, biopolymer processing has mainly adopted two strategies: traditional solution-based and more recent fibrillation-based methods (Figure 3d). For solution-based processing, solvent and salt are used to disrupt the complex hierarchical structure to produce biopolymer solutions. Using these solutions, various technologies have been developed to generate different material formats (e.g., particles, fibers, films, hydrogels, and sponges, ...
hydrogels). For fibrillation-based processing, the native biopolymer fibers are first downsized to fibers with length scales from nano- to micrometer sizes, through mechanical fibrillation assisted by chemical or enzymatic pretreatments. The isolated structures bear the native nanoscale building blocks, providing benefits of retention of these unique hierarchical and optimized structures, for subsequent fabrication into new materials with improved properties.

3. Solution-Based Biopolymer Processing: A Reverse Engineering Approach

Fibrous biopolymers like silk and cellulose are not soluble in water or in most common organic solvents. The insolubility originates from the inter- and intramolecular hydrogen bonding between the biopolymer chains. The hydrogens bonds promote the assembly of the biopolymers chains to possess high crystallinity, and to dissolve these biopolymers, various solvent systems have been developed. Traditional solvent system usually involves using harsh conditions like strong acid or base, or toxic chemicals to break the intermolecular hydrogen bonding. Developing solution-based biopolymer products in a sustainable way often requires the use of green solvents. This green solvent concept is proposed to address the environmental and health footprints with characteristics of low vapor pressure, easily recyclable and reusable, nontoxic, bioderived, rapid solubilization, and low temperature in polymer processing applications.[46] In this section, we focus mainly on aqueous-based silk processing and the recent advances in the use of green solvents in cellulose processing.

3.1. Aqueous Solution-Based Silk Processing

3.1.1. Silk Structure

Natural silkworm (B. mori) fibers have a core–shell structure: two parallel fibroin fibers with a triangular cross-section coated with a layer of sericin. The silk fibroin core is semicrystalline and responsible for the excellent mechanical properties of silk fibers. Sericin serves as a glue to hold the two fibroin fibers together and responsible for the excellent mechanical properties of silk with a layer of sericin. The silk fibroin core is semicrystalline with nanosized crystallites embedded in an amorphous matrix. Silk fibroin consists of bundles of interlocking nanofibers ≈30 nm in diameter.[48] Within each nanofiber, the silk molecules assemble into a network structure, with nanosized β-crystallites embedded in an amorphous matrix. Silk fibroin consists of a heavy chain (390 kDa) and light chain (26 kDa) linked together by a disulfide bond at the C-terminus, forming a H-L complex.[45] This H-L complex binds a glycoprotein, P25 (30 kDa), in a ratio 6:1 via hydrophobic interaction to form an elementary micellar unit.[46] At the molecular level, the amino acid sequence of the H-chain is comprised of a repetitive core region and nonrepetitive C- and N terminal regions. The repetitive core region consists of 12 hydrophobic and 11 hydrophilic domains, which form the crystalline and amorphous regions in the silk fiber, respectively. The amino acid sequences of the hydrophobic domain mainly consist of Gly-X (65% Ala, 23% Ser, and 9% Tyr).[45] with (Gly–Ala–Gly–Ala–Gly–Ser) forming the antiparallel β-sheet structures.[31,47] The β-sheet structures stack together to form β-crystallites by hydrophobic interactions/van der Waals interactions between the sheets. The hydrophilic domains assemble into random coils and helical structures; responsible for the flexibility of the silk fibers.

Silk polymers are reported either based on crystalline structure (e.g., silk I and silk II)[36,48,49] or protein secondary structure (e.g., β-sheet, β-turn, α-helix, and random coil). The secondary structure and crystallinity are determinants of the physical properties of both natural silk fibers and regenerated silk materials, including mechanical, thermal, optical, and dielectric properties. The main reason for the fast-expanding use of silks in advanced fields such as regenerative medicine, drug delivery, optics, and electronics originates from the ability to control the polymorphs of silk to enable the fabrication silk materials with different properties, thus a broadening range of applications.

3.1.2. Aqueous-Based Silk Processing and Functionalization

The extensive inter- and intrahydrogen-bonding among silk chains results in limited dissolution options, mainly lithium bromide/H2O,[47] CaCl2/CH3CH2OH/H2O,[50] hexafluoroisopropanol (HFIP),[51] N-methylmorpholine N-oxide (NMMO)/H2O and ionic liquid (IL).[52] These solvents dissolve silk at the molecular level, resulting in complete destruction of the silk hierarchical structure and degradation of the silk fibroin molecules. Among these solvents, dissolving degummed silk fibers in 9.3 M LiBr solution has been the most successful and widely used protocol to prepare aqueous silk solution, providing a material platform for downstream processing to generate useful silk-based materials. As a chaotrope salt, lithium bromide at high concentration breaks the intra- and intermolecular bonds to enable the dissolution of silk fibers. The typical LiBr bromide protocol involves three major steps: degumming, dissolution, and dialysis. In the degumming process, silk cocoon pieces are boiled in Na2CO3 solution for typically 5 to 60 min to remove the sericin. In addition to sericin removal, the degumming process causes silk degradation due to cleavage of the disulfide bond between the fibroin H-chain and L-chain and fragmentation of the amorphous silk sequences in the fibroin.[53] Without degumming, sericin would induce silk aggregation and only very low concentrations of silk aqueous solution are obtained. After drying, the degummed silk fibers are dissolved in 9.3 M LiBr solution until complete dissolution, followed by dialysis against deionized water to remove the LiBr and then centrifugation/filtration to remove any debris. The final result is an aqueous silk solution; defined as regenerated silk solution (Figure 4a). This solution is normally stable at 4 °C for a few weeks to months depending on the processing conditions. Compared to native silk fibroin, the regenerated silk aqueous solution preserves the primary amino acid sequence and can assemble into the secondary structures found in native fibroin. However, the silk fibroin often has a reduced and broad range of molecular weights as a result of the processing.[54] This change in molecular weight between the native protein and...
the purified and solubilized protein affects the refolding and reassembly into materials and the physical properties of these materials.

The regenerated silk fibroin solution has been used to generate a spectrum of material formats including particle, fibers, films/membranes, hydrogels, and 3D objects. Using manufacturing techniques ranging from simple film casting to advanced techniques such as EBL and IBL, strategies to manipulate the refolding of regenerated silk molecules, including physical crosslinking, chemical crosslinking, enzymatic crosslinking, and templated-assembly (Figure 4c), permit the formation of multidimensional silk materials with different secondary structures, crystallinity, and higher order structures (Figure 4d–l).

Another appealing feature of this all-aqueous silk processing is that it supports the ability to add in dopants with the aqueous silk solution to fabricate silk materials with desired active functions (Figure 4b), including enzymes, growth factors, antibodies, nucleic acids, nanoparticles, quantum dots, etc. Specifically, biomolecules are often susceptible to denaturation in organic solvent, so the use of toxic organic solvents, such as HFIP, formic acid, and methanol should be minimized during silk processing. The all-aqueous silk processing conditions allow for the incorporation of labile chemical and biological components that retains their activity for extended time frames. Extensive work has been reported on the ability of silk to stabilize various bioactive compounds (e.g., growth factors, enzymes, antibodies, small molecules) as a function of adsorption, covalent attachment, entrapment, and/or encapsulation. Thus, a versatile silk-based toolkit is currently available for encapsulation, entrapment and sustained drug delivery formulations, from small molecules through complex macromolecular enzymes and therapeutics.

### 3.1.3. Regenerated Silk Materials

The ability to reverse engineer silk fibroin into aqueous solutions represents a platform for the development of appropriate material formats to meet requirements for many applications. Aside from its traditional use for textiles, the applications of silk have been extended to other fields such as regenerative medicine, drug delivery, optics, and electronics by taking advantages of its compelling mechanical properties, biocompatibility, biodegradability, optical transparency in the film format, as well as the facile incorporation of functional components.

#### 3D Silk Scaffolds: 3D porous silk scaffolds can be prepared via a number of approaches, including freeze-drying of frozen aqueous silk solutions, salt leaching from aqueous solution, and 3D printing to generate scaffolding materials for in vitro and in vivo tissue engineering and regeneration. Silk scaffolds have been successfully used for the long-term culture of cortical regions of the brain (Figure 5a), adipose, intestine, kidney, skin, and bone tissues, among others. These in vitro constructed healthy and disease tissue models can provide platforms to understand disease progression and potentially serve as substitutes for animal models for drug testing. In addition, injectable porous silk scaffolds...
microparticles have received FDA approval for tissue augmentation in vocal cord repair and regeneration (Figure 5b).[70]

Silk Hydrogels: Silk hydrogels have been mainly used as carriers for the delivery of therapeutics, as matrices for 3D in vitro tissue models, and as fillers for in vivo tissue repair cell delivery.[71–73] Many methods induce silk gelation, including physical, enzymatic and chemical crosslinking.[74] Typically, physically crosslinked silk hydrogels form via sol–gel transitions, where β-crystallites form and act as physical crosslinkers to hold hydrogel networks together. The formation of physically crosslinked silk hydrogels can be accelerated and controlled using different environmental factors including pH, salts, temperature, nonsolvents, vortexing, sonication, and electric fields. Injectable silk gels for localized delivery of chemotherapy drugs due to their ability to maintain high concentrations of drugs at the tumor site without the need for surgical implantation or systemic administration.[72,73] For example, sonication-induced silk hydrogels loaded with Dox were injected locally to breast tumors in mice, resulting in significant reduction in primary tumor growth and metastasis when compared to equivalent doses of Dox administered systemically (Figure 5c).[72]

Silk Nanoparticles: Silk nanoparticles can be prepared from regenerated silk solution by self-assembly and by controlling environmental factors including pH, salt concentration, and solvent composition.[75] Salting out can be used to prepare silk nanoparticles with a 500–2000 nm diameter range using a salt bath; factors including pH, salt, ionic strength, and silk concentration affect particle morphology, structure, yield, and dispersity.[76] Some solvents are also used to prepare silk nanoparticles, including methanol, ethanol, acetone, and dimethyl sulfoxide, where silk fibroin undergoes a structural transition from random coil to β-sheet during nanoparticle formation.[75,77,78] Other approaches include electrospray[79] and capillary-microdot technique.[80] Silk nanoparticles have been used to encapsulate chemotherapeutics and provide sustained drug release, resulting in increased plasma retention time, increased cellular uptake, drug targeting to tumors.[77,80–82] For example, when subject to magnetic field, drug-loaded magnetic silk nanoparticles accumulated at the tumor site and significantly suppressed the growth of multidrug resistant tumors (Figure 5d).[82]

Silk Films: Silk films have been widely explored for use in drug delivery,[6,8] tissue engineering,[84] wound healing,[85] optics,[86] and electronic devices.[87] Silk films are easily processed via drop-casting, spin-coating, dip coating, and layer-by-layer assembly.[63,86,87,89] Topographical features can be introduced onto silk films by various patterning techniques, and a
variety of post-treatment methods, including water-annealing, methanol treatment, and physical stretching to induce β-sheet formation, can be used to tune molecular structure and mechanical properties of the films. Silk films are of particular interest for optical applications due to their transparency, low surface roughness, nanoscale processibility, and ease of processing. A variety of photonic structures, including diffraction gratings, microprisms, microlens arrays, and inverse opals (Figure 5e–g) can be obtained by casting aqueous silk solution onto patterned substrate.[90,99] Silk films can also be used as substrates or active components in the fabrication of electronic systems.[87,91] Compared to conventional polymer film substrates, the robust mechanics, biocompatibility, biodegradability, biore sorbability, aqueous processing, and biochemical functionalization support silk films as a versatile platform for sustainable electronics.[87] Silk films with controllable degradation rate are candidates for fabricating transient electronics (Figure 5h).[89] Silk films functionalized with silver nanoclusters were used as switching layers to fabricate memristor (Figure 5i), to provide significant enhancement in switching speed, good switching stability, low set/reset voltages,[92]

3.2. Solution-Based Cellulose Processing

3.2.1. Cellulose Structure

Cellulose crystallinity influences the physical properties of the processed materials such as mechanical strength, thermal stability, and optical properties. Thus, it is important to understand the structural transformations during cellulose dissolution and regeneration in order to choose processing conditions for target material properties. Cellulose consists of a linear chain of β-(1→4)-linked d-glucose units. The anhydroglucose unit is the repeat unit that contains three reactive hydroxyl groups, a primary one at position 6 and two secondary ones at positions 2 and 3. Cellulose adopts different crystalline structures originating from the orientation of the hydrogen bonds between and within cellulose chains. Native cellulose exhibits cellulose I structure, with two different polymorphs: \( I_a \) and \( I_c \). Dissolution with strong base or ILs interrupts native cellulose I structure, yielding regenerated cellulose typically with type II structure.[93,94] The conversion of cellulose I to cellulose II is often irreversible, with cellulose II the more thermally stable phase. Generally, regenerated cellulose with cellulose II structure has inferior mechanical properties compared to native cellulose with cellulose I structure.[95] Structurally, the difference between cellulose I and cellulose II are the hydrogen-bond patterns in the crystalline structures; cellulose I has a parallel orientation of the macromolecular chains, whereas cellulose II has an antiparallel arrangement. From density functional theory modeling, cellulose I chains show a right-handed twist, while for cellulose II, \( (010) \) and \( (020) \) models twist in the opposite directions with right- and left-handed chirality.[96]

3.2.2. Green Solvents for Cellulose Dissolution

Although it has been more than 180 years since the first successful cellulose extraction by Anselme Payen in 1838,[97] cellulose dissolution remains a difficult process because of the relatively long cellulose molecular chains and the closely packed crystalline structures with a high density of intra- and intermolecular hydrogen bonding.[16] The first cellulose dissolution dates to 150 years ago with a mixture of \( \text{HNO}_3/\text{H}_2\text{SO}_4 \), leading to the invention of cellulose nitrate.[98] Since then, a huge variety of cellulose solvents have been used, including the viscose process, which involves the conversion of cellulose to cellulose xanthogenate by reacting cellulose with \( \text{NaOH} \) and \( \text{CS}_2 \).[99] The metastable cellulose xanthogenate is then used in aqueous sodium hydroxide as a spinning dope to form regenerated cellulose fibers. This process uses toxic chemicals and strong base, thus, not sustainable. With a sustainable focus, four non-derivative solvent systems are reviewed here, \( \text{NaOH}/\text{urea} \) system, NMNO system, ILs, and natural deep eutectic solvents (DESs). The physical dissolution of cellulose in these solvent systems is environmentally friendly with the goal of avoiding consuming chemicals since most of the reagents maybe recycled and reused, with no accompanying chemical reaction. Water-based system—\( \text{NaOH}/\text{urea} \) system—\( \text{NaOH} \) can disrupt the intermolecular hydrogen bonds of cellulose when there is only \( \text{NaOH} \) aqueous solution.[100] \( \text{NaOH}/\text{urea} \) precooled (−12 °C) aqueous solution can rapidly dissolve cellulose within 2 min.[101] The dissolution mechanism at low temperature differs from traditional methods, as the \( \text{NaOH} \) hydrates attract interactions with cellulose chains by forming new hydrogen-bonded networks, while urea hydrates surround the \( \text{NaOH} \)—cellulose as a shell structure to form an inclusion complex with a sheath like structure, leading to cellulose dissolution.[102] NMNO system—NMNO has been used as an organic solvent to dissolve cellulose since 1969.[103] The dissolution of cellulose using NMNO is a physical process, as NMNO is an excellent hydrogen bond acceptor as a result of strong N–O dipoles, which can break existing hydrogen bonding between cellulose chains.[103] The NMNO-based solvent can be recovered >99% in the Lyocell process, and there is no formation of harmful by-products.[104] However, the process has a relatively narrow temperature processing window as cellulose solution in NMNO undergoes a strong exothermic reaction above 120 °C, leading to extensive cellulose degradation.[105] ILs—ILs are efficient solvents to dissolve cellulose.[106] The beneficial features include negligible vapor pressure, high thermal and chemical stabilities, high solvating capabilities, structure tunability, easy recyclability, and nonflammability.[106] ILs are able to dissolve cellulose include imidazolium, pyridinium, ammonium-based cations and halides, carboxylate, and alkylphosphosphate-based anions.[107] Anions in ILs form strong hydrogen bonding with the hydrogen atoms on the hydroxyl groups of cellulose, while cellulose dissolution mechanisms are not completely understood.[108] ILs systems for cellulose dissolution are considered green although not fully industrialized. The life cycle analysis of cellulose dissolution in IL 1-butyl-3-methylimidazolium chloride (Bmim Cl) and NMNO solvents showed that Bmim Cl was a promising alternative to NMNO with similar environmental impact.[109] Recycling ILs is essential for using ILs as green solvents for cellulose dissolution, thus, thermoreversible ILs with easily induced phase separation are better options than low volatility choices. DESs—DESs have gained interest as
potential green solvents for cellulose dissolution because they are easy to prepare, low cost, and relatively low toxicity. These options are viewed as a lower-cost alternative to ILs. The first DESs were a system composed of a mixture of Lewis or Bronsted acids and bases with a variety of anionic and cationic species. Although DESs have a lot of similarities as ILs, they are a “designer solvent” as the physiochemistry properties can be tuned based on the constituents.

3.2.3. Regenerated Cellulose Materials

The preparation of regenerated cellulose materials involves cellulose dissolution and subsequent shaping and coagulation to form new material formats (Figure 6a,b). Most commercial cellulose products are regenerated cellulose through the viscose or lyocell process for fiber/film production used in textiles, hygienic disposals, and home fabrics. The viscose process is

![Figure 6. Cellulose dissolution, regeneration, material formats, and advanced applications. a) Illustration of cellulose dissolution from feedstock using green solvents and the dissolution mechanism of cellulose in ionic liquids. Reproduced with permission. Copyright 2017, the Royal Society of Chemistry. b) Shaping and regeneration of cellulose materials from cellulose solution with the structural changes and the various shaping and regeneration methods, including solvent evaporation to form films, chemical or physical crosslinking to form hydrogels, spinning to form cellulose fibers, and spinning drop atomization to generate cellulose microbeads. c) Examples of material formats of regenerated cellulose. Fibers. Reproduced with permission. Copyright 2007, Wiley-VCH. Films. Reproduced with permission. Copyright 2019, American Chemical Society. Microbeads. Reproduced with permission. Copyright 2005, American Chemical Society. Hydrogels. Reproduced with permission. Copyright 2007, Wiley-VCH. d) Examples of advanced applications of regenerated cellulose. Conductive regenerated cellulose films. Reproduced with permission. Optical cellulose fiber. Reproduced under the terms and conditions of CC BY license. Optical fibers. Reproduced with permission. The Authors. Published by Springer Nature. Wearable electronic textiles. Reproduced with permission. Copyright 2019, the Royal Society of Chemistry.](image-url)
based on derivatization, where cellulose is dissolved in solvent systems like NaOH/CS₂, and acetic anhydride. The Lyocell process is a direct spinning process based on cellulose dissolution by NMMO. The Lyocell process has less environmental impact because of the 99% reusability of the solvent, the physical dissolution mechanisms (instead of the chemical reaction of the viscose process), and no harmful by-products or reagents used.²⁰³

Regenerated cellulose-based materials like films, microbeads, and hydrogels are potential replacements for current petroleum-based materials to address some sustainability problems (Figure 6d). Current industrial uses of cellulose films made from viscose and cuprammonium processes focus on food casting, cosmetics, and medical/pharmaceutical packaging because of the biodegradability and renewability.²¹² However, these manufacturing processes are still complicated and expensive. More efforts have recently been directed toward generating cellulose films in low temperature solvents. The resultant films can reach good tensile strength of 100 MPa. Blending nanocomposites, such as cellulose whiskers in the system, also significantly enhanced the elastic modulus to 5 GPa. Besides mechanical properties, good oxygen permeability, biodegradability, and optical transparency were achieved, toward applications of the cellulose films as packaging materials.²¹⁶

Microbeads prepared from cellulose or cellulose derivatives are candidates for drug delivery,²¹¹ chromatography,²¹⁴ water treatment,²¹³ catalysis,²¹⁶ and protein immobilization.²¹⁷ Celullose has long been used in pharmaceutical industry as a drug excipient. Cellulose microbeads provide many advantages as drug carriers over conventional granulated materials, including high internal surface area,²¹⁸ tunable porosity, and facile manufacturing methods. The abundance of hydroxyl groups on the surface also enables chemical functionalization for specific drug loading. For water treatment, cellulose microspheres with nanoporous structures can be used to absorb toxic chemicals or metal ions via physical interactions.²¹⁶ Cellulose microbeads are also suitable candidates to replace persistent plastic microbeads in personal care products.²¹⁹

Cellulose-based hydrogels are useful in tissue engineering, drug delivery, wound healing, and other healthcare products due to their low cost, hydrophilicity, biocompatibility, and biodegradability. Currently, there are three types of network structures for regenerated cellulose hydrogels, chemical crosslinking, physical crosslinking, and chemical–physical double crosslinking. The network structures are highly relevant to the mechanical properties of these hydrogels.²¹⁵,²¹²

In recent years, regenerated cellulose-based materials have been developed toward more advanced applications (Figure 6d). The abundance, renewability, environmentally sustainability, and easy to make composite or functionalize makes it a good candidate for applications in energy storage, thermoelectric device, organic electronics, flexible electronics, and biosensors.²¹¹ Lv et al. have developed a method to transfer regener ated cellulose yarns into multifunctional wearable electronic textiles by coating the lyocell processed regenerated cellulose with polypyrrole. The material was stitched into a fabric, forming flexible textile micro-supercapacitors.²¹² Regenerated cellulose can also be used to prepare optical devices. Optical cellulose fibers have been prepared from regenerated cellulose by coating the cellulose core with cellulose acetate. These optical cellulose fibers demonstrated utility as water sensors.²¹³ Flexible and transparent conducting films were also fabricated by encapsulating silver nanowires between the regenerated cellulose film and poly(3,4-ethylenedioxythiophene)-polystyrene sulfonate) nanosheets. This new type of material can be used as strain-to-electricity sensors.²¹²

4. Fibrillated Biopolymers: Maintaining Native Structures as Building Blocks

As reviewed for silk and cellulose, biopolymer dissolution in combination with macromolecular reassembly is routinely used to form new biopolymer-based materials. However, dissolution is destructive to the evolutionarily optimized hierarchical structures, resulting in loss of their original and desirable properties. Although the biopolymers can reassemble into ordered structures during the regeneration of new materials, the complete restoration of native hierarchical structures is not achieved; thus a loss of native properties. Moreover, solution-based approaches involve complicated processing steps both during and after material formation, adding time, cost, and dissipative for large-scale, advanced manufacturing. In some instances, harsh or toxic chemicals are also used in solution-based polymer processing, in conflict with sustainability.

Instead of dissolving biopolymers and using regeneration-based methods to prepare biopolymer-based materials, new approaches for the direct utilization of natural building blocks in native biopolymer fibers for materials fabrication has been pursued with success. By retaining these inherent structural features, materials with exceptional properties have been generated. In this section, we summarize the top-down strategies for the fibrillation of cellulose and silk fibers to generate building blocks in the nano- to micrometer range and the assembly of these structures into functional materials. The methods utilized continue to be refined, with considerable efforts to improve efficiency, consistency, and sustainability.

4.1. Fibrillated Cellulose: Fibrillation, Assembly, and Applications

Fibrillated cellulose refers to cellulose fibers that are broken down into smaller fibers. Cellulose nanofibers (CNFs) and cellulose nanocrystals (CNCs) (Figure 7a) have raised extensive interest due to their high aspect ratios, strong mechanical properties, abundance, renewability, and biocompatibility, all driven by the retention of the native structural organization found in these materials as natural fibers.

4.1.1. Preparation of CNFs

CNFs refer to long nanofibrils with high aspect ratios (≥1000), with diameter of 3 to 100 nm and length of micrometers.²¹⁶ At the nanoscale, CNFs consist of alternating crystalline and amorphous regions stabilized by hydrogen bonds. The most common method for CNF preparation is by mechanical treatment (Figure 7b), including high-pressure homogenization,²¹⁷ microfluidization,²¹⁸ grinding,²¹⁹ cryocrushing,²²⁰ high
Figure 7. Preparation of nanocellulose. a) Illustrations and TEM images of CNF (left) and CNC (right). Reproduced under the terms and conditions of CC BY license.[153] Copyright 2018, The Author(s), Published by MDPI. b) Mechanical treatment for CNF preparation including high pressure homogenization, high-intensity ultrasonication, grinding, and microfluidizer. c–e) Pretreatment methods including chemical, enzymatic, and IL treatments are combined with mechanical treatment for CNF preparation. f) Chemical treatment to prepare CNCs through oxidation and acid hydrolysis. The dots indicate new functional groups through chemical reaction. g) Enzymatic treatment to remove amorphous domains to make CNC. h) IL pretreatment to swell cellulose fibers for further chemical treatment to make CNC. i) Mechanical treatment to remove amorphous regions to make CNC, including ball milling, ultrasonication, and high pressure homogenization.
intensity ultrasonication,\textsuperscript{[111]} and steam explosion.\textsuperscript{[112]} The mechanism is to break interfibrillar hydrogen bonding through mechanical forces, where the level of these forces and the types of mechanical treatment significantly affect the morphology and properties of the CNFs. However, high production costs for tools and materials, and energy input, are required to break the hydrogen bonding. The mechanical treatment also typically results in low solid density aqueous dispersions of CNFs, leading to transportation and storage difficulties.\textsuperscript{[113]}

Current studies have focused on green production of CNF by using chemical or enzymatic pretreatment to reduce energy input requirements. By introducing negative surface charge onto cellulose via TEMPO (2,2,6,6-Tetramethylpiperidinyloxy or 2,2,6,6-Tetramethylpiperidine 1-oxyl) oxidation (Figure 7c),\textsuperscript{[134]} mechanical treatment becomes easier with lower energy consumption. In addition, oxidation modifies surface cellulose functional groups from hydroxyl to carboxylic acid groups, to facilitate dispersion in water, and to increase potential for further modifications via more homogeneous reactions. A variety of other surface chemical modification methods can also be used to generate functional groups and to facilitate nanofibrillation.\textsuperscript{[19]} Enzymatic hydrolysis cleaves glycosidic linkages within the amorphous regions of the cellulose, producing new cellulose chain ends that facilitate fibrillation (Figure 7d). There are also enzymes that can selectively modify the carbohydrate network by oxidation, inserting negative charges onto cellulose chains to facilitate fibrillation.\textsuperscript{[115]} In addition, ILs can assist the process by swelling the cellulose chains before mechanical treatment as a more sustainable approach (Figure 7e).\textsuperscript{[136]} Pretreatment can also be achieved by DES,\textsuperscript{[137]} organic acid hydrolysis,\textsuperscript{[138]} and periodate.\textsuperscript{[139]}

\subsection*{4.1.2. Preparation of CNCs}

CNCs typically range in size from 3 to 20 nm (width) and 100 to 300 nm (length), depending on the origin of the cellulose and the preparation conditions. CNC is produced by chemical, mechanical, or enzymatic treatment of cellulose (Figure 7f-i), where the amorphous regions are removed, leaving largely rod-like CNCs.\textsuperscript{[140]} Acid hydrolysis is the prevalent process to prepare CNCs, which is achieved by treatment with concentrated sulfuric acid.\textsuperscript{[141]} When hydrolyzing and oxidizing the amorphous regions of cellulose to generate CNC, sulfuric acid also esterifies the surface of CNC to produce sulfate groups in the C6 position (sulfated CNC). Although this process has been used at industrial scale for CNC production, it has drawbacks including high energy consumption, low yield, and health and environmental hazards from the concentrated acid. Some challenges also limit the use of sulfated CNC, such as their hydrophilic feature, their tendency to aggregate in high ionic strength environments and their lack of thermal stability. Other acids are also used to hydrolyze cellulose to yield CNC, including hydrochloric acid,\textsuperscript{[142]} hydrobromic acid,\textsuperscript{[143]} phosphoric acid,\textsuperscript{[144]} or a mixture of nitric acid and acetic acid.\textsuperscript{[145]} In recent years, nonacid hydrolysis methods have been explored. In particular, extremely high densities of surface carboxylic acid groups can be achieved through TEMPO\textsuperscript{[146]} and ammonium persulfate oxidation.\textsuperscript{[147]}

More sustainable methods have been developed in recent years for CNC fabrication. Mechanical methods, including ultrasound,\textsuperscript{[148]} high energy bead milling,\textsuperscript{[149]} ball milling, and high-pressure homogenization,\textsuperscript{[166]} were developed to reduce the use of acids or oxidation agents. IL-assisted nanocellulose production was also developed.\textsuperscript{[136]} ILs have advantages over conventional methods, including 1) low vapor pressure and good thermal and chemical stability, making solvent recovery and reuse easier, 2) tunability of ILs in terms of chemical composition to provide a variety of pathways for nanocellulose fabrication with different morphologies depending on the anions and cations used. ILs can also be used simultaneously to modify the surface of the material, resulting in new chemical properties. However, toxicity and cost issues need to be considered for future industrial production.\textsuperscript{[136]} Similarly, DES can be used as a pretreatment to assist CNC production,\textsuperscript{[151]} while enzymatic hydrolysis provides a mild method for CNC production without the need of extra chemical reagents. The enzymatic method can also selectively hydrolyze the amorphous domains of cellulose without compromising the surface structure of the crystalline regions, facilitating further chemical modifications.\textsuperscript{[152]}

4.1.3. Preparation and Applications of CNF- and CNC-Based Materials

Nanocellulose is easily dispersed in water and forms colloidal systems due to the particle sizes and the surface charges generated by hydrolysis/oxidation methods. Water-based drying methods offer low cost, sustainable, and industrially mature options to assemble these biopolymer-based materials into new material structures. Because of the water-based processing, it is also straightforward to add new bioactive functionalities or to generate composite materials.

Figure 8 summarizes the applications of nanocellulose based materials including hydrogels, films/membranes, microbeads, and fibers. Nanocellulose-based hydrogels are good candidates for biomedical applications including 3D cell culture, wound dressing, tissue engineering scaffolds, biosensing, and drug encapsulation and delivery (Figure 8a–c).\textsuperscript{[154]} Besides hydrogels, nanocellulose based films have emerged as high performance materials with enhanced intrinsic characteristics including optical, mechanical, thermal, barrier, and insulating properties.\textsuperscript{[155]} With current available film casting methods, it is also reasonable to incorporate new functionalities like fire-retardants, as well as magnetic, electrical, and conductive properties into nanocellulose-based films, enabling applications from conventional paper-based to high-tech fields like photonic devices, thin film transistors, clean energy, biosensing, and smart membranes for environmental needs (Figure 8d–f).\textsuperscript{[155]} Nanocellulose-based microbeads have also emerged as new types of materials, with applications to replace microplastic beads, as drug delivery vehicles, and for water treatment (Figure 8g,h). Compared with regenerated cellulose microbeads, nanocellulose-based microbeads provide more tunability in terms of size, porosity, mechanical properties, and functionality through the various fabrication methods. Nanocellulose can also be used as the basic building blocks to fabricate cellulose fibers with exceptional mechanical properties. The composite fibers
with new functionalities can also be built based on nanocellulose.[156] For example, chitosan–CNF composite fibers were fabricated for wound healing.[157] Using a water-based spinning method, CNF and poly(diallyldimethylammonium chloride) were mixed to prepare composite fibers for advanced fibrous materials, including crimping and responsive smart textiles (Figure 8i,j).[158]

### 4.2. Fibrillated Silk: Fibrillation, Assembly, and Application

Inspired by the advances in the isolation of nanocellulose by top-down fibrillation approaches, the same methodology is being applied for the isolation of silk fibers in the nano to micrometer range from natural or degummed silk fibers (Figure 9a). The isolation of silk nanofibers (SNFs) was first demonstrated by downsizing native silk fibers into nanofibers with diameters of 25–120 nm using high intensity ultrasound.[160] The fibers formed aggregates and mats with poor dispersibility, limiting assembling into useful material formats. Recently, many chemical treatment methods have been developed for silk fiber fibrillation and they are often used in combination with physical treatments such as ultrasonication, high pressure homogenizer, and ball milling (Figure 9b). One strategy involves the incomplete dissolution of silk fibers using silk solvents, including formic acid (FA)/CaCl2,[170] HCl/FA,[171] FA/LiBr,[172] CaCl2:CH3CH2OH:H2O,[173] and HFIP.[174–176] These solvents partially destroy the hydrogen bonds and leave the nanofibrils structures intact, depending on time and temperature of treatment, resulting silk fibers with sizes of 20–200 nm in diameter. NaOH/urea, an efficient solvent for dissolving cellulose and chitin, was recently used to treat degummed silk fibers by destroying the van der Waals forces between molecular layers, resulting in nascent silk nanoribbons composed of single β-sheet layers and amorphous silk molecules.[177] Sodium chlorite solution treatment integrated with varying levels of energy input dissociated silk fibers into different structures, including microfibrils, nanofibrils, nanorods, and nanoparticles.[178] SNFs with lengths and widths of 306 ± 107 and 4 ± 18 nm were obtained through ultrasonication-assisted sulfuric acid hydrolysis processing.[179]

However, some limitations are associated with these chemical-based strategies, including expensive, harsh, and toxic solvents, long processing times and short stabilization times for the fiber suspensions, raising sustainability concerns and hindering scale-up. Some efforts have been made to improve sustainability of silk fibrillation. As a green and chemically tailorable solvent, different types of DES were integrated with sonication or high-pressure homogenization to generate SNFs.[180,181] DES attacks the amorphous regions of natural silk fibers and exfoliated silk fibers into millimeter/micrometer-scale segments, which were further processed into nanofibers by mechanical disintegration. This process generated SNFs with diameters ranging from 2.3 to 28 nm and lengths of 598 ± 308 nm at yields >70% under optimal DES pretreatment conditions. Without using any harmful chemicals, a scalable approach involving a sequential process of milling, agitated bead milling, heating, and high-pressure homogenization was developed to generate SNFs from degummed *B. mori* fibers.[182] The resultant fibers were generally 200–300 nm in width with diameters of 2–4 μm. In comparison to the microgram scale production and chemically intensive processing of other reported methods, tens of grams of SNFs were produced per batch without using additional chemicals.

The isolated silk fibers at nano- to microscales can be used as building blocks for processing into various material formats, such as fibers (Figure 9c),[176] films (Figure 9d),[173–175,177,180,181,183,184] inks (Figure 9e)[180] and hydrogels,[183] for applications including filtration, regenerative medicine, drug delivery, and electronic devices. The fibrillated fibers can also be used directly for mechanical reinforcement due to their robust mechanical properties.[177,180] Compared to the abundance of silk processing technologies developed using reverse engineered, unfolded, solution-based silk molecules as the starting materials, processing fibrillated silk fibers obtained via top-down approach into useful materials is still in early research phases.

High performance silk fibers were obtained by dry spinning using a dope of silk microfibril solution (Figure 9e) prepared by partially dissolving degummed silk fibers into microfibrils.[176] The as-spun fibers maintained structural hierarchy and the well-organized silk nanofibril structures of natural silks. Compared to the mechanical properties of natural silks, the modulus of the best performing fibers (8 ± 1 GPa) was higher than some spider and *B. mori* silks, while the strength (109 ± 34 MPa) and toughness (13.9 ± 9.2 MJ m⁻³) were still significantly lower. The yarn-like spiral fibers produced by rotating the collector on a plane perpendicular to the fiber axis supported human dermal fibroblast growth along the direction parallel to fibrillar orientation. As a common technique to fabricate films or membranes from suspensions of silk micro-/nanofibers,[173–175,177,180,181,183,184] vacuum-infiltration was used to assemble liquid-exfoliated SNFs into thin silk films with and without patterned structures (Figure 9d). In addition, conductive silk membranes were obtained by adding conductive reagents, such as single- or multiwall carbon nanotubes,[177,183] into the system. For example, composite membranes of SNF-multiwall carbon nanotubes were used for wearable electronics to detect human motions such as finger bending, blinking, pulsing, speaking, swallowing, and coughing.[183] The amphoteric SNFs obtained by DES treatment can serve as an “ink” to be directly written on negatively charged TEMPO-oxidized cellulose nanofibers (TOCN) and positively charged partially decetylated chitin nanofiber membranes (DEChN) (Figure 9e), showing potential for anticounterfeiting devices, silk-based packaging, and smart composites.[180]

### 5. Fibrous Biopolymer Thermal Processing

Thermoplastic processing offers an inexpensive, simple, and fast method for material processing. In fact, the solid-based (solvent-free) processing method represents a more sustainable approach under the guideline of 12 principles of green chemistry,[185] as it prevents waste and avoid the possible use of hazardous solvents. However, it remains a challenge to process biopolymers with hierarchical structures via thermal-based methods because the temperature gap between thermal transition and thermal degradation is too small to allow
Figure 9. Preparation, assembly, and application of fibrillated silk fibers. a) Examples of fibrillated silk fibers in the form of microfibers, nanofibers, and nanoribbons. Microfiber. Reproduced with permission.[178] Copyright 2018, Wiley-VCH. Nanofiber. Reproduced with permission.[180] Copyright 2020, American Chemical Society. Nanoribbon. Reproduced with permission.[177] Copyright 2018, American Chemical Society. b) Chemical and mechanical treatments to prepare fibrillated silk fibers. c) Silk microfibers prepared by partial dissolution of degummed B. mori fiber were assembled into fiber bundles through dry spinning. 3D cell patterns generated on yarn-like spiral silk fibers. Fluorescent images show preferential alignment of human dermal fibroblasts (green) along the axes of the regenerated silk fibers (red). Reproduced under the terms and conditions of CC BY license.[176] Copyright 2016, The Authors. Published by Springer Nature. d) SNFs prepared by liquid exfoliation was assembled into silk membranes. SEM images and photos (from left to right): Cross-sectional SEM image of the liquid exfoliated SNF membrane at low and high magnifications. Photo of an SNF membrane with excellent transparency. CdSeS/ZnS quantum dot patterned SNF membranes prepared via masked vacuum-filtration under UV light. Photo of gold single crystal nanoplatelet patterned SNF membranes prepared by vacuum filtration of SNF suspensions through a vacuum filtration membrane with a predeposited gold single crystal nanoplatelets pattern. Photo shows SNF based flexible electronic devices deformed with the deformation of a pig ear. Reproduced with permission.[175] Copyright 2016, Wiley-VCH. e) SNFs prepared by treatment of DES solvent assisted by mild mechanical disintegration. TEM image shows SNF morphology. SNF ink directly written on TOCN and DEChN membranes. Photos of SNF-written TOCN membranes before (top) and after (bottom) immersion in Ponceau S solutions. Photos of SNF-written DEChN membranes before (top) and after (bottom) immersion in toluidine solutions. Reproduced with permission.[180] Copyright 2020, American Chemical Society.
thermal-based processing. Currently, the thermal processing of biopolymer often involves heavy use of plasticizers or chemical modification to bring thermoplastic properties to the biopolymer, which often lead to inferior mechanical properties in end products when compared to the native biopolymer. In this section, we first discuss the recent progress in silk thermal processing with the aim of inspiring new routes for biopolymer thermal processing. We then summarize the state of art of cellulose thermal processing.

5.1. Thermoplastic Molding of Silk

Silk is considered as a nonthermoplastic fiber due to its high content of β-sheet structure as well as its complex hierarchical structure.[186] The β-sheet crystalline structures are so stable that attempts to melt them have normally resulted in decomposition which obscures the melting process.[17,48] An exception is that β-sheet crystalline structures can melt by fast laser heating upon input of heat energy,[13] which suggests a useful way for thermally processing β-sheet-enriched biomaterials. However, in addition to the high cost, technical challenges remain on how to scale up to process bulk materials. Therefore, the existence of β-sheet crystalline networks in native silk fiber structure is considered the major hurdle in silk thermal processing. To circumvent this issue, efforts have been made to use silk materials with low content of β-sheet as starting material for thermal processing.

5.1.1. Thermoplastic Processing of Regenerated Silk Films: Plasticizing Effect of Water Molecules

The as-cast silk films from regenerated silk solution are normally noncrystalline with a very low content of β-sheet structure.[17] Variable Tgs have been reported for amorphous silk films cast from aqueous silk solution, ranging from ≈180 °C for dried silk films to approximately room temperature for films subjected to high humidity (>90%) treatment.[17,187] As a plasticizer, water molecules can enhance the chain mobility in the noncrystalline domains of proteins by disrupting the intermolecular cohesive forces between protein chain and reducing steric hindrance for movement and reorientation.[40,188] Thus lowering the Tg. When the amorphous silk film is heated above its Tg, silk fibroin molecules enter a viscoelastic liquid state prior to crystallization. With the assistance of pressure ≈50–80 psi, silk patterning, imprinting and welding can be realized (Figure 10). [189–192]

The nanoimprinting of silk films can be performed at different temperatures, depending on the water content of the material (Figure 10a,b).[189] Silk films prepared at ambient humidity of ≈35% have a Tg ≈ 100 °C. A nanopattern of a heated hard master can thus be imprinted on the silk film at 100 °C. When silk films are saturated with water, their Tg drops to room temperature, and thus can be imprinted at room temperature. Similarly, PiP can be achieved by using a dried crystallized silk film as a master.[193] The nanopatterns on dry crystallized silk film can be directly transferred to a wet noncrystallized blank silk film (Figure 10d–g). The flexibility of the crystallized silk films allows the conformal imprinting on nonplanar surfaces master. Compared to nanoimprinting, PiP significantly increases throughput by duplicating the patterns using each generation to imprint the next. Spatially controlled welding between silk fibroin laminates was achieved through the addition of a spatially patterned amorphous silk layer in between the crystallized silk films to be adhered followed by heat pressing (120 °C, 80 psi (Figure 10h–k)). Once heat and pressure are applied, the amorphous layer enters a viscoelastic liquid state and welds the outer films together.[190]

5.1.2. Thermoplastic Molding of Regenerated Silk Materials

Lyophilized silk, featured by its amorphous structure, is a shelf-stable silk materials format for on-demand use. For example, lyophilized silk stock of moderate to low molecular weight can be fully reconstituted even after 1 year of storage at elevated temperature (37 °C).[193] Using lyophilized silk powder as raw material, a new thermal processing method was developed to transform solid-state regenerated silk directly into a robust, dense, structural material with tunable mechanical properties, while retaining good machinability, biocompatibility, and biodegradability.[21]

Solid-state silk thermal processing involves two steps: the production of amorphous silk nanoparticles (ASN, diameters from 30 nm to 1 μm) and processing of ASN by thermal pressure (Figure 11a). Fresh regenerated silk solution prepared via the well-established LiBr dissolution method is diluted, lyophilized, and milled to obtain ASN. Wide-angle X-ray scattering, Fourier-transform infrared spectroscopy, and solid-state NMR spectroscopy analysis demonstrate the ASNs are primarily amorphous in structure, with a high content of random coil or helical structures and <1% of β-sheet. The ASN powders are then packed in a predesigned mold, followed by hot pressing at high pressure (632 MPa), leading to densification and fusion of the silk nanomaterials into bulk materials, such as plates, cylinders, and bars. The silk bulk materials are stronger than those obtained by solvent-based processing and have strength when compared to most natural structural materials (for example, wood) and to some synthetic materials (Figure 11b).[21] The silk bulk materials have excellent machinability and can be processed into medical devices with complex shapes (e.g., silk bone screws and ear tubes) (Figure 11c–f). Further, the molecular structure and physical properties of the resulting materials can be tuned by controlling the molecular structure of the starting materials and processing parameters utilized, including temperature, pressure, and plasticizer. Another advantage of silk thermal processing is its compatibility with bioactive molecules such as enzymes and antibiotics, which can be blended into the silk aqueous solution before lyophilization or mixed with lyophilized silk powder (ASN) in the solid state. For example, protease XIV, an efficient protease to degrade silk fibroin,[194] can survive the heat pressing process and maintain activity even at temperatures to 145 °C.[21] Incorporating silk-degrading enzymes into the constructs that activate upon hydration offers a novel tool to tune silk degradation for in vivo applications.
Figure 10. Thermoplastic processing of regenerated silk films. Illustration of the two nanoimprinting processes: a) hot embossing and b) room-temperature embossing. c) SEM image of silk film imprinted with a periodic array of chromium nanoparticles (200 nm diameter and 30 nm height) separated by 250 nm. (a–c) Reproduced by permission.[189] Copyright 2010, Wiley-VCH. d) Illustration of the protein–protein imprinting (PiP) and replication mechanism. The pattern in a crystallized silk fibroin master (yellow) was transferred to an untreated, unpatterned film by pressing the two stacking layers on a heated substrate at 120 °C for 60 s, with pressure of about 50 psi. SEM images showing the original pattern of the master film and the inversed pattern...
The novel combination of heat, pressure, and water as plasticizer guides the self-assembly of the amorphous silk fibroin molecules in the solid state, enabling silk thermal processing (Figure 11h). Regenerated ASN powders have a well-defined water-associated low $T_g$. Silk-based bulk materials were formed by direct thermal fusing the ASN under high pressure at temperatures above $T_g$. Temperature is a key factor controlling the molecular structure and crystallinity of the silk bulk materials generated in the process. When the temperature is high enough, silk molecules gain sufficient mobility to overcome the energy barrier for structure transition, forming intra- and intermolecular $\beta$-sheet nanocrystalline structure. The kinetics of silk crystallization is similar to isothermal cold crystallization of polymers. When the temperature is just above the $T_g$, the crystallization rate is slow, resulting in low crystallinity in the final material. In the higher temperature range, crystallization rate increases significantly and a higher crystallinity can be achieved. The presence of bound water in ASN (5.0 ± 0.5 wt%), is critical for the structure transition during silk thermal processing; removal of the bound water in ASN by preheating results in the failure of silk thermal processing. The heat-treated ASN can regain thermal processibility by moisture treatment.

Three different processing routes were developed to fabricate silk-based bulk materials: HFIP-based, sol–gel–solid, and thermal. For HFIP-based method, lyophilized silk powders were dissolved in HFIP, followed by long-term methanol treatment to induce $\beta$-sheet structure. Silk bulk materials were then obtained after extensive drying to remove the HFIP and methanol. For the sol–gel–solid based method, controlled evaporation of water from aqueous silk solution resulted in a sol–gel-solid transition and silk bulk materials were obtained. In comparison to solvent-based processing to prepare silk bulk materials, silk thermal processing is advantageous due to the shortened processing time and improved efficiency (Figure 11i). A cost, time, energy, and environmental impact assessment for the three processing methods to make silk bulk materials demonstrated that the thermal processing method has the shortest time, lowest cost, and least waste generation, while also offering on-demand production from the ASN (unlike the other methods). Thermal processing using the lyophilized silk (ASN) as source material allows the fabrication of silk-based bulk materials within an hour. In addition, the long shelf-life of ASN circumvents the limitations of solution-based processing methods to ensure reproducibility and scalability. Using tools widely used in commercial polymer processing, silk thermal processing to fabricate solid silk materials represents a significant step toward industrial production of silk-based materials.

5.2. Cellulose Thermal Processing

Due to the abundant hydrogen bonding and crystalline fibrous structure, direct thermoplastic molding of pure cellulose is difficult. Efforts have been made to disrupt hydrogen bonding to facilitate thermal processing of pure cellulose materials. For example, a combination of mechanical shear, pressure and laser irradiation was used to disrupt the hydrogen bonding, resulting in thin transparent cellulose disks. This process had high energy demand and was too harsh for industrial production. Ball milling of cellulose powder can also be used to disrupt the hydrogen bonding network, and the direct molding of bulk plastic-like cellulose materials was realized using back pressure-equal channel angular pressing at 150 °C without additives. However, the mechanical properties of the resulting bulk cellulose material had a storage modulus of 1 GPa, significantly lower than that of native cellulose. Therefore, thermal processing of pure cellulose to generate materials with compelling mechanical properties remains challenging.

Adding plasticizers can facilitate the thermoplastic molding of cellulose. For example, Bmim Cl has been used as a plasticizer to facilitate thermal processing of microcrystalline cellulose. Although molding of bulk cellulose was achieved, the mechanical properties were significantly lower than those of native cellulose due to the high content of plasticizer. IL-plasticized cellulose containing 30 wt% Bmim Cl had a Young’s modulus of 200.7 MPa and tensile strength of 8.3 MPa, while native cellulose with a cellulose I structure has a Young’s modulus of 138 GPa and tensile strength of 13–17 GPa.

Other efforts toward cellulose thermal processing focus more on chemical modifications to introduce side chains to the cellulose main chain. Chemical modification to convert hydroxyl groups to esters, ethers or other functional groups improves the mobility of the cellulose chains for thermoplastic properties (Table 2). Thermoplastic cellulose esters can be prepared by using short chain acids such as nitric acid, acetic acid, and propionic acid. The first commercial products of thermoplastic cellulose derivatives were celluloid, consisting of cellulose nitrate with camphor added as a plasticizer. Cellulose acetate and short chain aliphatic esters such as cellulose acetate propionate and cellulose acetate butyrate represent commercially important thermoplastic cellulose esters widely used in making fibers, films, coating, and molded plastics. However, thermal processing of these classic cellulose esters was challenging because the $T_g$s and the flow temperatures are close, resulting in a narrow processing window for thermoplastic molding. Therefore, large amounts of plasticizer, such as glycerin, polyethylene glycol, and triethyl citrate are generally added to facilitate thermal processing. However, the diffusion of plasticizers...
into and out of the system significantly weaken performance and decrease the lifetime of cellulose plastics. Adding long-chain aliphatic esters to cellulose could be a strategy to enhance thermoplastic processibility, as the fatty acid can disrupt the hydrogen bonding and also be retained longer in the bulk as a plasticizer.[203] For example, cellulose octanoate (C8) and cellulose palmitate (C16) are thermally processible without using plasticizers.[204] However, chemical modification with long chain fatty acids usually requires high temperature, long reaction times, catalysts, and/or special acylation to enhance reactivity.[205] These harsh conditions often cause significant degradation of the cellulose and makes the process expensive and less efficient. Ester substitutes containing both bulky moieties and soft segments can also be introduced to improve the mobility of the cellulose chains.[201] The resulting cellulose esters can be processed into various shapes by traditional melt
processing techniques such as injection molding, melt extrusion, and hot pressing without adding plasticizers. Another strategy to enhance cellulose thermoplastic processability is by grafting polymers as plasticizers. Examples include grafting polylactic acid\(^{206}\) and polycaprolactone\(^{207}\) onto cellulose. However, a high grafting ratio is required to achieve suitable

Table 2. Thermoplastic cellulose derivatives.

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<td>Cellulose acetate</td>
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<td>Cellulose acetate propionate</td>
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<tr>
<td>Cellulose acetate butyrate</td>
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<tr>
<td>Cellulose acetate phthalate</td>
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<tr>
<td>Cellulose ester with long chain aliphatic groups</td>
<td></td>
<td>[202,209]</td>
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<tr>
<td>Cellulose ester with various bulky groups</td>
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<tr>
<td>Methyl cellulose</td>
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<tr>
<td>Hydroxyethyl cellulose</td>
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<td>[210]</td>
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<tr>
<td>Hydroxypropyl cellulose</td>
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<td>Carboxymethyl cellulose</td>
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<tr>
<td>Cellulose acetate-g-poly(lactide)</td>
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<tr>
<td>Cellulose acetate/butyrate-g-poly(caprolactone)</td>
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<td>[207,208,211]</td>
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<tr>
<td>Cellulose acetate-g-poly(methacrylate)</td>
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thermoplasticity, resulting in materials with decreased thermal stability and increased fragility.

6. Chitin Processing

Chitin is structurally similar to cellulose: cellulose consists of α-glucose units connected by β-(1→4) linkages, while chitin contains repeat units of 2-(acetylamino)-2-deoxy-d-glucose (Figure 1b,d). Owing to the chemical and structural similarities between cellulose and chitin, their processing techniques also share some common features. Similar to cellulose, solution processing is the preferred technique to prepare chitin-based materials (e.g., fibers, beads, films, and hydrogels), including the use of green solvents like ILs and NaOH/urea system.[211] For chitin fibrillation, many processing techniques employed for cellulose fibrillation have been transferred to chitin fibrillation, including the three main methods: mechanical, chemical modification combined with mechanical, and strong acid hydrolysis. This topic has been extensively reviewed elsewhere.[19,20,212]

The direct thermoplastic molding of pure chitin or its derivatives without plasticizers has not been reported, while the chemical modification of chitin through acylation has been extensively explored to improve processability.[213] However, it remains challenging to thermally mold acylated chitin alone due to the closeness of the decomposition and flow temperatures, resulting in a narrow processing window for thermoplastic molding. In addition, the thermal molding of chitosan (deacetylated chitin), the most common derivative of chitin, has been achieved through adding plasticizers and applying thermomechanical treatment (mechanical kneading).[214] This process can be a potential alternative to the traditional casting methods for chitosan film production at large scale.[215]

7. Challenges and Perspective

Synthetic polymers are petroleum-derived materials, most often processed using simple, low cost thermal methods to form plastics and related materials that provide major benefits to society. However, these benefits come at an increasing cost related to sustainability and environmental impact. By contrast, many abundant natural fibrous biopolymers are remarkable in structure and function, and have been utilized in many applications, but without the broad utility seen with synthetic plastics, but that potentially can impact sustainability. This disparity is in large part driven by the processing complexity of fibrous biopolymers in contrast to the simplicity for synthetic polymers, mainly due to the complex structural hierarchy that drives the unique structure-function of native biopolymer structures, but challenges the use of thermal processes that are essential to the widespread utility of synthetic polymers. Thus, a key need is to develop thermal processing modes with fibrous biopolymers to match the simplicity found with plastics processing, while also being cost competitive and preserving the benefits of sustainability. Thus, new opportunities to process native natural materials toward new functional material outcomes with more efficient approaches are required. Two major paths toward these goals were summarized in this review.

1) New insights into thermal processing of fibrous biopolymers (e.g., silk, cellulose) to reduce solution processing requirements and to simplify approaches for consistency and cost-competitiveness, while maintaining sustainability. 2) Fibrillation, whereby the native structural components are preserved for further use downstream, thus shortening and simplifying top down processing steps to achieve improved efficiency as well as sustainability.

There remain many major challenges ahead to maintain this recent momentum in the use of fibrous biopolymers toward broader utility, with sustainability goals in mind.

Mechanisms of Thermal Processing—Continued insight into thermal processing methods, not only with silk but with cellulose and chitin, to improve feedstock utility to reduce solvent requirements and to avoid the need for derivatization. Enlightened by the success with silk thermal processing, future work should focus on applying the same methodology adopted for silk thermal processing to other fibrous polymers such as cellulose and chitin. We envision that the similarities in structural hierarchy and thermal stability between the various fibrous biopolymers suggest a path forward with most of these systems.

Molecular Modeling—Modeling of structural hierarchy of fibrous biopolymers can guide experimental methods toward more efficient approaches and predictive outcomes, such as for processing windows. Revealing protein behavior under high temperature and pressure improves understanding of mechanisms underlying thermoplastic processing of protein-based materials. In addition, modeling the effect of various processing parameters (e.g., temperature, pressure, plasticizers such as water and glycerol) would provide important guidance on sustainable additives for the process. The ultimate goal is to create an optimal protein-based thermoplastic processing environment that advances softening and structural transformation while avoiding significant decomposition at the processing temperatures. The modeling can similarly be applied to cellulose and chitin toward the same outcomes.

Scale Up—Scaling is needed to increase outcomes and to identify process controls and to identify issues with the scaling early on as these new methods are developed. These needs apply to thermal processing as well as to the fibrillation approaches.

Bioactive Functionalization—The need is to continue to exploit the unique ability of inherent biopolymer chemistries to facilitate the incorporation, stabilization, and function of bioactive additives to the thermal processes (e.g., enzymes, antibodies, therapeutics, etc.), to generate new functionalized biopolymer-based plastics. One appealing feature of silk thermal processing is the ability to retain the activity of some bioactive additives even at high temperature (90–175 °C) and pressure (>600 MPa), which enables the generation of biopolymer-based plastics with tailored functions. Future work should explore stabilization options for other fibrous biopolymer systems (e.g., cellulose and chitin) during thermal processing.

The future is bright. It took over 60 years to move silk processing from solution methods into the thermal processing world. However, with these new insights, new impetus in sustainability, and with new scientific motivations toward these goals, there is substantial potential for us to positively impact
polymer options into the future, with sustainability as a key component in decision making.

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Conflict of Interest

The authors declare no conflict of interest.

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