Recent advances in nanoengineering cellulose for cargo delivery

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Abstract

The recent decade has witnessed a growing demand to substitute synthetic materials with naturally-derived platforms for minimizing their undesirable footprints in biomedicine, environment, and ecosystems. Among the natural materials, cellulose, the most abundant biopolymer in the world with key properties, such as biocompatibility, biorenewability, and sustainability has drawn significant attention. The hierarchical structure of cellulose fibers, one of the main constituents of plant cell walls, has been nanoengineered and broken down to nanoscale building blocks, providing an infrastructure for nanomedicine. Microorganisms, such as certain types of bacteria, are another source of nanocelluloses known as bacterial nanocellulose (BNC), which benefit from high purity and crystallinity. Chemical and mechanical treatments of cellulose fibrils made up of alternating crystalline and amorphous regions have yielded cellulose nanocrystals (CNC), hairy CNC (HCNC), and cellulose nanofibrils (CNF) with dimensions spanning from a few nanometers up to several microns. Cellulose nanocrystals and nanofibrils may readily bind drugs, proteins, and nanoparticles through physical interactions or be chemically modified to covalently accommodate cargos. Engineering surface properties, such as chemical functionality, charge, area, crystallinity, and hydrophilicity, plays a pivotal role in controlling the cargo loading/releasing capacity and rate, stability, toxicity, immunogenicity, and biodegradation of nanocellulose-based delivery platforms. This review provides insights into the recent advances in nanoengineering cellulose crystals and fibrils to develop vehicles, encompassing colloidal

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nanoparticles, hydrogels, aerogels, films, coatings, capsules, and membranes, for the delivery of a broad range of bioactive cargos, such as chemotherapeutic drugs, anti-inflammatory agents, antibacterial compounds, and probiotics.

Graphical Abstract
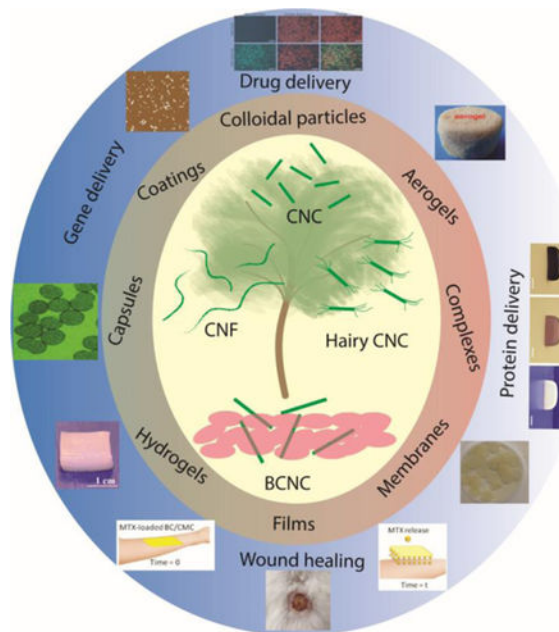
Keywords

Nanocellulose; cellulose nanocrystals; hairy nanocellulose; bacterial cellulose; cellulose nanofibrils; drug delivery; wound healing; cancer therapy

Introduction

Cellulose is the most abundant biopolymer in the world, which is made up of linear glucose rings connected to each other through β(1 → 4) glycosidic bonds [1]. Bengt G. Rånby, whose early observations in 1951 pioneered the field of nanocelluloses [2], noted that the strong acid treatment of cellulose fibers almost always resulted in the formation of nanoscale needle-like colloidal particles (initially called micelles). The breakdown of cellulose fibers into dispersed nanowhiskers with width ~ 7 nm and varying length based on the degree of polymerization provides an indirect evidence for heterogeneity and the hierarchical structure of cellulose fibrils. This observation attests to the coexistence of regions prone to acid-mediated hydrolysis and regions resistant against the chemical modification. Later, this was attributed to the difference in crystallinity along the cellulose fibrils made up of alternating regions of crystalline and amorphous (disordered) regions [3]. The crystalline regions are difficult to penetrate; therefore, the reactants can only modify its surface, eventually resulting in the dissolution of the outer layer, whereas the amorphous layers are readily accessible [4] for facile chemical modification [5–9].

Nanocelluloses may be categorized into four main groups: cellulose nanofibrils (CNF), cellulose nanocrystals (CNC), hairy CNCs (HCNC), and bacterial CNCs (BCNC). Cellulose fibers undergoing rigorous mechanical shear experience defibrillation, yielding nanoscale fibrils with dimensions in the range of several nanometers in diameter and up to tens of microns in length, named cellulose nanofibrils [10–13]. Chemical modification of amorphous chains may be conducted through strong acid hydrolysis to remove the amorphous regions, resulting in CNC with width ~3–70 nm (typically less than 10 nm) and length ~ 25–500 nm (typically between 100200 nm) regulated by the fiber source [7]. The structure of CNF resembles spaghetti with a higher aspect ratio than rice grain-like CNCs. Recently, hairy cellulose nanocrystals have been produced by oxidizing and solubilizing the amorphous regions of fibrils. Periodate oxidation of fibers yields dialdehyde-modified cellulose fibrils, which can be heated at 50 ℃ to increase the solubility of modified amorphous biopolymers. The disintegration of these fibrils into CNCs sandwiched between two layers of protruding dialdehyde cellulose chains yields neutral hairy cellulose nanocrystals, also known as sterically-stabilized cellulose nanocrystals. In another scenario, when the dialdehyde-modified cellulose fibrils are further oxidized with chlorite at room temperature, the fibrils readily fall apart in the solution, yielding CNCs attached to highly dicarboxylated protruding cellulose chains at both ends. This family of nanocelluloses has been named anionic hairy cellulose nanocrystals or electrosterically stabilized cellulose nanocrystals [8,9]. Another type of cellulose named bacterial cellulose (BC) is produced by organisms, such as bacteria [14,15]. BC is typically produced as fibers, which may chemically be treated by strong acids and disintegrated into nanocrystals known as bacterial CNC [16]. BC typically attains a high degree of crystallinity and longer crystalline body than conventional CNCs produced from wood fibers. Figure 1 summarizes nanocelluloses and their common synthesis methods.

Nanocelluloses play promising roles in a broad range of applications [20,21], spanning from water treatment [22–24], environment and energy [25–27], catalysis [28], optics and photonics [29–31], sensing and biosensing [32], and biomedicine [33–38]. There was a limited literature on cellulose-based nanomedicine before 2014, which mainly focused on nanocellulose- and dissolved cellulose-base colloidal dispersions, colloidal hydrogels, and films for binding various types of molecules, such as chemotherapeutic agents to decrease their diffusion rate. Small-scale structural features of nanocelluloses have been the focus of research for developing drug delivery systems. In addition, biocompatibility and cytotoxicity [39] have sometimes been evaluated when new combinations of molecules have been added to nanocelluloses.

Here, we focus on the state-of-the-art nanocellulose-based cargo delivery systems, which have mainly emerged since 2014. The articles before 2014 are briefly summarized in each section, and relevant review papers are highlighted to provide a brief overview on the early cellulose-based nanomedicine research. We thoroughly review the recent advances in the synthesis, fabrication, and functionalization of biomaterials based on cellulose nanocrystals (non-hairy and hairy), bacterial cellulose, and cellulose nanofibrils for the delivery of a wide range of cargos, such as drugs, proteins, and antimicrobial agents and highlight various forms of nanocellulose-based delivery systems, including hydrogels, films, and aerogels. This paper provides a comprehensive review with a forward-looking approach on the

nanocellulose-mediated cargo delivery platforms for nanomedicine, which have emerged in the past four years.

1. Nanocellulose properties

Physical, mechanical, chemical, and biological properties of nanocelluloses have been thoroughly reviewed in the literature. Here, we briefly explain the main characteristics of nanocelluloses to emphasize how this class of nanomaterials may be beneficial in biomedical applications, particularly for the controlled release of active substances. Depending on the cellulose source, key properties of nanocelluloses, such as shape, size, and crystallinity may vary [7,40–42]. Nanocelluloses sourced from organisms, such as bacteria and algae, are typically more pure (free from other polysaccharides, such as lignin and hemicellulose) and have a higher crystalline index (>90%) than those produced from plants (<70%) [5,43]. Accordingly, cellulose sources regulate the properties of nanocelluloses, such as density, porosity, hydrophilicity, and water absorption. An interesting example is porous *Cladophora* (green algae) cellulose with an extremely large surface area (> 100 m^2 g^{-1}) [44] compared to plant-derived nanocelluloses [45]. The aspect ratio (length-to-width ratio) of CNFs (which can be > 500) is noticeably higher than cellulose crystals (CNC, HCNC, or BCNC, typically < 200), while the width of all four types of nanocelluloses is typically in the order of 5–10 nm [5].

Hydrogen bonding plays an important role in the mechanical properties of nanocelluloses. According to the theoretical calculations, when hydrogen bonding is taken into consideration, the longitudinal modulus of cellulose I ~ 173 GPa, whereas it reduces to ~ 71 GPa without intramolecular hydrogen bonding [46,47]. Accordingly, hydrogels and aerogels [48] prepared from nanocelluloses typically benefit from high mechanical strength. Besides mechanical properties, the rheological characteristics of nanocelluloses are important in biomedical applications. Nanocelluloses have unique rheological properties, regulated by their surface chemistry, morphology, and colloidal ordering (e.g., liquid crystalline and gel states) [40,45,49–55]. At high enough concentrations, nanocelluloses form physical hydrogels, which are typically shear-thinning at particle concentrations lower than the critical phase transition to an ordered suspension [56].

The key contributions of nanocelluloses to nanomedicine would not be possible without the chemical modification of CNCs, HCNCs, BCNCs, and CNFs to modulate their functionality and the effective binding of target compounds for controlled release. An overview of the chemical reactions of nanocelluloses has been provided in literature [57], highlighting oxidation as one of the most common modification methods. By oxidizing the hydroxyl groups, which may be followed by reductive amination and esterification reactions, bioactive compounds may be covalently attached to nanocelluloses. Self-assembly of nanocelluloses is another, often less noticed, method to bind active molecules. For example, Varjonen *et al.,* [58] and Valo *et al.,* [59] attached cargos such as beclomethasone dipropionate (BDP) and itraconazole (ITR) to nanocelluloses using genetically engineered binding protein HFBI-DCBD composed of hydrophobin and showed their stability for several months.

Besides chemical conjugation of cargos, some chemical treatments, such as TEMPO (2,2,6,6tetramethylpiperidine-1-oxyl)- or chlorite-mediated oxidation may alter the nanocellulose charge, promoting the physical bonding of charged molecules to the carrier. In addition, HCNCs, decorated with highly functionalized cellulose polyelectrolytes, provide nanocarriers with tunable surface charge [60], which are able to regulate the chemical responses to carried drugs. Surface charge may have negative effects on the attached cargo, e.g., degrading aspirin over time [61]. The crystalline structure of HCNCs can be preserved by controlling the oxidation reaction condition. As innovations in nanocellulose-based therapeutics advance, thorough *in vitro* and *in vivo* characterizations are required to ensure that the product is a reliable molecular carrier for clinical applications.

In vivo behavior of nanocelluloses is rarely explored. Current literature, geared towards investigating the adhesion, proliferation, spreading, viability, and differentiation of a variety of cells in nanocellulose scaffolds, has attested to the biocompatibility of nanocelluloses when their concentrations are typically less than 1 mg mL^{-1}. CNCs are ingestible and their gastrointestinal fate is associated with acid-mediated hydrolysis and interactions with gut flora [62]. Importantly, concentration-dependent apoptotic toxicity of CNFs at 2–5 mg mL^{-1} has been reported [63]. Cationic nanocelluloses, e.g., trimethylammonium-CNF have been reported to be less cytotoxic than the anionic counterparts, e.g., carboxymethylated CNF [64]. Similar to nanocelluloses, BC is non-toxic against endothelial and osteoblast cells [65–67]. Toxicological studies of nanocelluloses have been conducted based on *in vitro* multi-trophic assays, acute lethal tests, gene mutations, and chromosomal tests, as well as oral evaluation, neurocytotoxicity, and immunoreactivity. Both CNF and BC may contain endotoxin and (1,3)-β-D-glucan contaminants, confirmed by analyzing the hot water extracts of these nanocelluloses using ELISA assays, high-performance size exclusion chromatography-multiangle laser light scattering, gas chromatography, and nuclear magnetic resonance spectroscopy [68].

2. Cellulose nanocrystals (CNC and HCNC) and their composites for cargo delivery

Here, we discuss the contributions of CNCs to the controlled release of active molecules, including a brief insight into the potentials of HCNC, a newly emerged class of nanocelluloses, in nanomedicine. CNCs, sourced from plants, are rod-like particles with length ~ 100–200 nm, width ~ 5–10 nm, and a hydroxyl-rich surface, facilitating a wide range of chemical modification [59]. These nanocelluloses are being commercially produced in large scales (hundreds of tons per year). We review the recent advances in CNC-based cargo delivery and bioimaging.

The focus of CNC research for biomedical applications before 2014 was geared towards taking advantage of the mechanically strong crystalline body of CNC for reinforcing composite biomaterials [70–72]. For example, Wang and Chen utilized cellulose nanowhiskers to improve the mechanical properties of electrospun prolamin protein fibers and create a drug delivery system that could prolong riboflavin release in phosphate buffered saline (PBS) [73]. Controlled release of water-soluble drugs, such as doxorubicin (Dox) and

tetracycline from unmodified CNC as well as hydrophobic anti-cancer drugs, such as docetaxel, paclitaxel, and etoposide from cetyltrimethylammonium bromide (CTAB) modified CNC have been demonstrated [74]. Xiang *et al.,* [75] modified CNC and demonstrated that the diffusion rate of non-ionic molecules could be controlled by adding electrospun poly(lactic acid) (PLA) to the matrix, which induced the Fickian diffusion with reduced initial (burst) release for Columbia blue dye as a model drug.

Despite the limited attempts for using CNC in biomedical engineering before 2014, the significant progress in synthesis, chemical modification, and production process optimization in the past four years has set the stage for the advanced medical applications of CNCs, in the forms of nanoparticles, hydrogels, films, and composite coatings. A summary of the main delivery applications of various forms of CNC-based biomaterials is presented in Figure 2. HCNC, however, has emerged recently, opening new horizons for producing CNCs that can accommodate ~ 1–7 mmol carboxylic acid groups on their surface [8,9], pushing the structural boundaries of conventional nanocelluloses. Applications of HCNC in the controlled release of active biomolecules has not been explored yet. The versatile chemical modality of this family of nanocelluloses may provide numerous possibilities for cargo loading and release.

2.1. CNC for cargo delivery

Viable intracellular cargo delivery platforms must be nontoxic towards somatic and undifferentiated stem cells and pass through the cell membrane [76]. CNCs are currently being explored as carriers for drug delivery, because they can be inexpensively produced from widely available renewable resources [13,69,77,78] and attain large surface areas (~ 100 m^2 per gram) [79] regulated by nano-morphology, particle size, and dispersity, tailored surface charge, and a high hydroxyl group content [19,42,69] for the physical and chemical binding of bioactive components. The bioactive components may be drugs for treating wounds or illnesses, or dyes to allow for visualizing the trajectory of carriers. Common drugs and contrast agents physically or chemically attached to CNCs are summarized in Table 1.

The fate of nanoparticles inside the body, regulated by protein adsorption, cellular uptake, and tissue translocation depends on numerous colloidal factors, including size, surface charge, and morphology [91,92]. Mechanisms of *in vitro* uptake and *in vivo* behavior of nanocelluloses as emerging platforms for cargo delivery have not been fully understood. Among the most important properties of nanocelluloses, surface charge plays a key role in regulating cellular uptake. Mahmoud *et al.,* studied the effect of ζ-potential (the potential difference between the nanoparticle surface and the bulk electrolyte) on the bioactivity of CNCs conjugated with commonly used dyes, fluorescein-5-isothiocyanate (FITC) and rhodamine-B-isothiocyanate (RBITC) [93]. ζ-potential, defined as the potential difference between the nanocrystals and the surrounding fluid environment had a direct effect on cell viability and cytotoxicity. CNC-FITC uptake by human embryonic kidney 293 (HEK-293) cells and *Spodoptera frugiperda* (*Sf9*) cells was sensitive to pH, peaking at pH ~ 5 and dropping drastically at pH ~ 6.5. HEK-293 and *Sf9* cells also absorbed CNC-RBITC mostly at pH ~ 5, and at the physiological pH, CNC-RBITC absorbance was much higher than

CNC-FITC. ζ-potential analysis revealed that both neat and FITC-tagged CNCs have negative surface charges, −31.6 mV and −46.4 mV, respectively, while RBITC-tagged CNCs have a positive surface charge (~ +8.7 mV) at this pH range [93].

Increasing CNC-FITC concentration from 30 to 300 μg/mL decreased cell viability from 59% to 29%, whereas cell viability was ~66% at 300 μg/mL of CNC-RBITC. Electric cell-substrate impedance sensing (ECIS) was conducted to study the cytotoxicity mechanism of FITC-tagged nanocrystals. Toxic compounds inhibited current flow on a gold electrode coated with *Sf9* cells cultivated with varying amounts of CNC, CNC-FITC, and CNC-RBITC. CNC and CNC-FITC inhibited the current flow with half-inhibition concentrations (ECIS$_{50}$) ~ 61 ± 2.4 μg/mL and 45 ± 4.6 μg/mL, respectively. ECIS$_{50}$ for CNC-RBITC was 224 ± 6.3 μg/mL, indicating that CNC-RBITC inhibited cell growth less than other tested nanocrystals [93]. While cellulosic nanocrystals, often decorated with negatively-charged moieties, are potential materials for targeted drug delivery, the cytotoxicity studies suggest that functionalizing nanocelluloses with positively-charged groups may improve their uptake and reduce the toxicity, which must be well understood before these nanoparticles can serve for clinical applications.

The interactions between CNCs and cargo molecules are regulated by chemical and/or physical modifications. Beside simple mixing, e.g., to produce CNC-alginate nanoparticles in honey (as a surfactant and stabilizer) for rifampicin delivery [94], a facile method to engineer the surface charge of CNCs is through the adsorption of polycations. Sulfuric acid-hydrolyzed negatively charged CNC surfaces can be coated with positively-charged polyethylenimine (PEI) to enhance small interfering RNA (siRNA) binding [87]. siRNA is a double-stranded RNA which is introduced into a cell to silence a specific gene. While siRNA serves as a potent method to alter various disease-causing agents, its susceptibility to enzymatic degradation and lack of cell target specificity are obstacles that require improvement. To this end, PEI has been used to facilitate siRNA delivery (Figure 3) via the high density of positively charged functional groups. This strategy not only enhances the electrostatic binding of siRNA to CNC but also improves the endocytosis and further enables endosomal escape as a result of the so-called "proton sponge effect" [87]. The high buffering capacity of the polycation increases the influx of counterions and water into the endosomes, disrupting the endosomal membrane due to elevated osmotic pressure, which results in releasing the cargo in the cytosol [95].

The formation of CNC-PEI has been confirmed by the change in ζ-potential from ~−40 mV to ~+37 mV [87]. Subsequent siRNA adsorption decreases ζ-potential to ~ +2.5 mV. The CNC-PEI-siRNA complexes have been assayed for cytotoxicity, cellular uptake, siRNA protection, and apoptosis induction [87]. Cytotoxicity assays reported no toxic effect of CNCs-PEI on the murine C2C12 myoblast cell line for certain doses and culture conditions within 72 h. Cellular uptake was evaluated by 4′,6-diamidino-2-phenylindole (DAPI) and ethidium bromide fluorescence, which showed the localization of siRNA complexes in the cytoplasm. The degree of siRNA protection was tested by exposing the CNC-PEI-siRNA to RNase A for a limited time followed by confirming the indicative bands of siRNA in gel electrophoresis after the separation of siRNA from the complex using sodium dodecyl sulfate. The gel assay demonstrated that siRNA was protected in the presence and absence of

RNase A. In addition, the presence of siRNA was not confirmed in complexed siRNA that was only exposed to RNase A, indicating that siRNA encapsulation is preserved even after the exposure to nuclease. Finally, the expression levels of extrinsic and intrinsic apoptotic genes, Casp 8 and Casp 9, respectively, were measured as indicators for apoptotic induction. Casp 8 was measured as an indicator of apoptotic induction via external stimuli (e.g., carrier attachment to the periphery of the cell) while Casp 9 was used to indicate the internal stimuli (carrier internalization). Casp 9 mRNA expression levels, measured by the quantitative analysis of cDNA produced from extracted mRNA, were significantly upregulated while Casp 8 expression was almost unchanged. This data supported that the CNC-PEI-siRNA was successfully endocytosed by the cells and delivered to the cytoplasm, followed by the siRNA-induced internal apoptosis (Figure 3) [87].

Drug delivery systems must preferably be nontoxic and stable in the circulatory system while remaining stealth against immune system. While carbon nanotubes [96] and self-assembled nanocarriers [97], such as lipid nanoparticles and vesicles [98] have been dealing with this challenge, CNCs with proper surface modifications may serve as a biocompatible biopolymer carriers for bioactive molecules. Hydroxyl groups on CNCs provide active sites for bioconjugation and promote hydrophilic interactions, decelerating phagocytosis. Needle-shaped nanoparticles, i.e., CNCs, of 50–200 nm length, may provide optimal evasion of both renal and immune system regulation [99].

Low cytotoxicity and uptake of unmodified CNCs are desirable properties for targeted drug delivery. It has been reported that CNCs are not cytotoxic against human brain microvascular endothelial cells (HBMECs) and have minimal uptake [99]. The viability of HBMECs, indicated by the formazan product of MTT assay, was not significantly different from the CNC-free control at CNC concentrations ~ 10, 25, and 50 μg/mL of media after 24, 48, or 72 h. Cellular uptake of FITC-tagged CNCs was time and dose dependent in 24 h exposure to 50 μg/mL of CNC; however, the uptake was minimal as the delivery system was non-specific. Because CNC uptake was minimal, these nanoparticles may be used as targeted drug delivery systems, which are able to minimize non-specific drug uptake.

Non-specific pharmaceutical cancer drugs (chemotherapeutic agents) typically cause damages to healthy tissues and organs. To limit these detrimental side effects, current research and development are driven towards creating specific targeting drug carriers using nontoxic materials. CNCs at optimum concentrations may not have toxic effects on the body and are not associated with specific cellular uptake. Additionally, the hydroxyl groups along the CNC body provide attachment sites for agents, such as fluorescent dyes, targeting materials, and therapeutic drugs. Typical chemical conjugation pathways rely on the facile oxidation, amination/amidation, esterification, etherification, and carbamation [100] of CNCs, enabling a broad range of surface modifications.

Targeted delivery may be achieved through the surface modification of CNCs with cell-specific receptors via amination. Targeting has been successful in brain tumor cells, whereby the targeting mechanism relies on folic acid (FA)/folate receptors that are overexpressed in cancer cells. Dong *et al.* [80] utilized these attachment sites to have CNCs bind specifically to three types of brain tumor cells: DBTRG-05MG and H4 (human brain tumor cells) and

C6 (rat brain tumor cells). By adding FA to CNCs tagged with FITC, the endocytosis of FITC-CNC-FA drastically increased. X-ray diffraction and Fourier-transform infrared spectroscopy showed that the crystallinity and crystal lattice structure of CNCs were not affected by attaching FITC or FA. All three brain tumor cells endocytosed the FITC-CNC-FA, where fluorescence intensities revealed that the uptake was 1472, 975, and 46 times greater than those of FITC-CNC for DBTRG-05MG, H4, and C6 cells, respectively. Further investigation revealed that DBTRG-05MG and C6 cells, both non-neuronal cells, operate on a different uptake mechanism than H4 ganglioma cells. Genistein, an inhibitor of caveolae-mediated endocytosis, inhibited 85% and 55% of FITC-CNC-FA uptake in DBTRG-05MG and C6 cells, respectively, but had no significant effect on the H4 uptake. Conversely, chlorpromazine, an inhibitor of clathrinmediated endocytosis, inhibited 48% of FITC-CNC-FA uptake in H4 human brain tumor cells. FA-mediated cellular uptake of CNCs has also been used for enhanced tumor ablation [101], wherein the selective enhancement of the electroporation-based therapy of human breast cancer cells is achieved via defect formation on the cell membrane as a result of CNC-FA endocytosis as well as the distribution of electrically non-conductive CNCs throughout the tissue. Figure 4 presents the enhanced cellular uptake of CNC-FA for targeted delivery.

Besides cell-specific receptors, recent studies of protein functions have revealed that albumins play a critical role in cell specific drug delivery through their specificity for ligand binding [102–107], which have been used as endogenous cargo carriers [108,109]. Common ligands for albumin include fatty acids, hormones, and toxic metabolites. The degree of cholesterol production from endothelial cells in the vascular arteries is a major factor that contributes to the formation of atherosclerotic plaques. In an attempt to enhance cholesterol efflux from these endothelial cells, the effect of cellular exposure to bovine serum albumin (BSA)/human serum albumin (HSA)-conjugated CNCs on cholesterol deposition was studied. CNCs were conjugated to each type of albumin in two ways: physical adsorption and chemical conjugation. Physically adsorbed BSA/HSA CNCs were prepared by direct addition and incubation of dried CNCs in BSA and HSA solutions overnight. Chemical conjugation was conducted through the TEMPOmediated formation of carboxylic acid groups on CNCs, followed by EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride)/NHS (N-hydroxysuccinimide)-mediated addition of BSA/HSA [88].

Both negatively and positively charged CNCs adsorb albumins. Binding between the cationic pyridinium-grafted CNCs and BSA is endothermic, which is driven by charge neutralization, whereas negatively charged (e.g., carboxylated and sulfated) CNCs show a weaker protein binding [110]. The efficiency of protein loading may be studied through the analysis of the supernatant after exposing CNC dispersions to BSA/HSA. Physical adsorption has yielded 40% and 36% loading efficiency while the chemical conjugation has led to 54% and 46% loading for BSA and HSA, respectively [88]. Interestingly, microscopic observations suggested that the topology of the CNC particles differed upon protein binding. Albumin-free CNCs were observed to have rod-like structures while albumin attached CNCs appeared to have more porous structures and loose arrangements so as to demonstrate a decent capacity to encapsulate various cargo. Secondary and tertiary structures along with general protein integrity were confirmed via SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and CD (circular dichroism) spectropolarimetry,

suggesting that CNC does not have adverse effects on the cargo proteins before or after binding. This was also supported by quantifying the bioactivity of proteins released from the CNCs, which reached up to 90% as compared to the activity of BSA/HSA in their native states [88]. Fluorimetric cholesterol efflux assays have revealed that while cholesterol efflux is constantly high in human coronary artery endothelial cells (HCAECs) independent of the concentrations and conjugation method, human umbilical vein endothelial cells (HUVECs) experienced a gradual increase in cholesterol efflux with increasing the concentrations of both BSA- and HAS-conjugated CNCs. Notable differences were observed for chemically-conjugated CNCs as opposed to the physically-adsorbed CNCs, whereby the chemically conjugated BSA/HSA demonstrated higher efflux. Accordingly, while albumins may serve as shuttles for cholesterol efflux, CNCs are also able to perform as sinks, carrying the effluxed cholesterol to the intestinal lumen and out of circulation.

Common surface modifications of nanocelluloses involve steps that demand organic solvents, which often renders these nanomaterials less biocompatible and more difficult to produce at large scales. To address these issues, ionic liquids have been designed to replace organic solvents. Ionic liquids are a desirable alternative due to their significantly low vapor pressure and ease of separation [111]. One-step esterification using Bronsted acid ionic liquid N-methylpyrrolidinium hydrogen sulfate (MePyrrHSO$_4$) [112] is an alternative to organic solvents. CNCs may chemically bind to chlorotoxin (Cltx) via a one-step esterification process, yielding Cltx-CNCs. Cltx is a protein that allows targeting brain tumors, such as glioblastoma multiforme (GBM) via specific binding to MMP-2/ClC3. Subsequent cellular studies using the U87MG GBM cell line shed light on the efficacy of Cltx-CNC as a target-specific drug delivery system against brain tumors [112].

Cytotoxicity of the functionalized Cltx-CNC was evaluated by an MTS assay upon the exposure of seeded cells to various concentrations of Cltx-CNC (up to 200 μg/mL) and compared to varying concentrations of non-functionalized CNCs [112], showing no significant toxicity. The internalization assay was conducted by observing the cellular uptake of Cy5-conjugated Cltx-CNC (Cy5-Cltx-CNC) via fluorescence staining. U87MG GBM, secreting high levels of MMP2 (matrix metalloproteinase 2), internalized the Cltx-CNC the most. Fluorescence staining of Cy5-Cltx-CNC indicated the nanoparticle localization in the cytoplasm and nuclei surrounding. On the other hand, MCF7 with low levels of MMP2 expression indicated no intracellular Cy5 fluorescence. The demonstrated biocompatibility and specificity for MMP2 targeting indicate the potential of Cltx-CNC as a brain tumor targeting drug delivery system.

CNC may also be used as a coating material for the enhancement of colloidal stability, biocompatibility, biodegradability, and chemical functionality of nanoparticles. Modified CNCs with tris(2-aminoethyl)amine (AMFC) have been used to coat Fe$_3$O$_4$ magnetic nanoparticles (AMFC-MNPs) (Figure 5) [82]. The nanocellulose was treated with tosylchloride for the functionalization with tris(2-aminoethyl)amine, which was selected to provide amino groups and cationic properties. AMFC was able to effectively bind to the carboxyl groups of methotrexate (MTX, an anticancer immunosuppressive drug), overcoming the downsides of MTX by maximizing its anticancer efficacy while minimizing the off-target side effects to healthy cells. While the drug-free AMFC-MNP showed a high

cell viability, the nanoparticles loaded with MTX had comparable cytotoxicity to free MTX, demonstrating that the efficacy of the bound drug is not inhibited by the nanoparticle. Hemolysis assays performed at 0–5000 μg/mL of blood showed less than 2.6% hemolytic activity for the drug-free AMFC-MNPs. The MTX-loaded nanoparticles had significantly higher cellular uptake than AMFC-MNP. This may be attributed to the chemical similarity of MTX to FA, facilitating receptor-mediated cellular internalization, which attests to the potential of MTX for enhanced cell-targeting (Figure 5) [82]. In the MTXAMFC-MNP system, drug encapsulation efficiency was ~ 91.2% along with a decent, 30.4%, drug loading efficiency. The pH-regulated controlled release is achieved based on the protonation states of MTX carboxylic groups. At physiological pH (7.4), up to 29% of the drug was released over 5 days, while up to 79% was released in acidic conditions (pH ~ 5.4). Magnetic CNCs have also been prepared using direct co-precipitation of Fe^{2+} and Fe^{3+} in CNC dispersions containing NaOH [113].

2.2. CNC hydrogels, foams, and complexes

One of the main motivations for using CNCs in drug delivery scaffolds is to benefit from the stiff body of nanocrystals (elastic modulus > 100 GPa [79]) to reinforce the otherwise mechanically weak carriers. Biocompatible and biodegradable polysaccharide-based nanodrug delivery systems, such as chitosan (CS), which often lack mechanical resilience may be reinforced with CNCs [114–116]. Chitosan-based bionanocomposites reinforced with CNCs for tissue engineering and drug delivery are among the most common delivery scaffolds [117–119]. Other non-cytotoxic biopolymers, such as xanthan gum (XG), which are able to provide controlled drug release, have been used as additives in the therapeutic scaffolds loaded with chemotherapeutic drugs, such as 5-fluorouracil (5-FU) [83]. To evaluate the *in vitro* performance of CNC-based drug delivery hydrogels, encapsulation efficiency, cumulative release, and cytotoxicity have mainly been evaluated.

Typically, both compressive strength and stiffness increase with increasing CNC concentration. For example, XG-CS had compressive strength and stiffness ~ 36 kPa and 1432 N/m, respectively, whereas for XG-CS-CNC, they ranged from 40 kPa-58 kPa and 1525 N/m-2398 N/m for 2–10% CNC concentrations [83]. The cumulative drug (5-FU) release was regulated by the CNC: while the CNC-free system released more than 75% of the drug within the first four hours, adding 10% CNC was able to slow down the kinetics and provide ~ 60% release in ~ 30 h. This may be explained by the increased structural rigidity and enhanced physical binding between the 5-FU and hydrogel scaffold induced upon the incorporation of CNCs. Cytotoxicity assays for the hydrogels indicated that the drug delivery system was not toxic against the cells (NIH/3T3), which was concluded based on more than 95% cell viability at all concentrations of CNCs used for the preparation of hydrogels. These results suggest that CNC-reinforced biocompatible nanocomposite hydrogels may provide controlled release platforms, benefitting from decent biocompatibility and improved mechanical and chemical properties.

Besides reinforcement, CNCs may impart hydrophilicity to polymeric matrices, enabling the dispersion of hydrophobic agents, such as curcumin (Cur) in aqueous media. Cur provides antioxidant, anti-inflammatory, and antibacterial activity, which may potentially be effective

for wound infection treatment; however, it is not trivial to maintain it in physiological systems due to its hydrophobicity. Porous collagen-cellulose nanocrystal (Coll-CNC) composites have been used to synthesize foam scaffolds loaded with curcumin-gelatin microspheres (GM) for prolonged Cur release [85]. This biomaterial has exhibited antibacterial activity in addition to dermis regeneration and inflammation inhibition as tested in mouse wound-infection models. The GM carriers embedded in the CNC scaffolds release Cur over a significantly longer period as compared to the GM or the CNC scaffolds alone [85], providing sustainable antimicrobial activity against various strains of bacteria. In the mouse model, the Cur/GM/Coll-CNC demonstrated accelerated dermal repair without significant wound hemorrhage or inflammation.

Increasing the dispersibility of drugs in aqueous media may not necessarily increase the circulation time in which case sudden increase in the drug concentration followed by its elimination significantly decrease the therapeutic effects and increase the side effects. Drug complex formation with nanoengineered polymeric networks may enhance the circulation time. Conventional oral drugs come into effect upon diffusion in the body fluids, such as intestinal fluid, followed by the uptake into the bloodstream. Oral drugs are known to have increased efficacy upon meeting certain criteria, among which drug residence time in the intestine is a key factor. Polyelectrolyte macroion complexes (PMC), synthesized from CNCs and chitosan have provided a long residence time in the small intestine due to the attraction between the negatively charged intestinal mucosa and positively-charged chitosan, providing an efficient platform for oral drug delivery [120].

2.3. CNC composite films

CNCs have typically been added to polymeric films to increase their Young's modulus, elongation at break, and dissolution/release time. CNCs have been incorporated into composite electrospun nanofibers for wound care and skin regeneration. Typically, CNCs are electrospun with polymers/nanoparticles and target drugs, such as poly (D, L-lactic-co-glycolic acid) (PLGA), Cur, and polyethyleneimine (PEI)-carboxymethyl chitosan (CMCS)/ plasmid DNA (pDNA)-angiogenin (ANG) [86]. ANG stimulates angiogenesis in wounded areas, facilitating skin regeneration by improving oxygen and nutrient delivery. Since ANG is highly unstable and has a short half-life in serum, drug delivery systems, such as chitosan-based nanoparticles, have been engineered to introduce ANG into cells via the transfection of pDNA. To examine the effectiveness of this nanofiber scaffold in skin regeneration, multiple tests have been conducted, including the measurement of pDNA release, ANG expression, and cell viability and proliferation, followed by *in vivo* evaluations using rat models [86]. PLGA/CNC/pDNA-ANG provided a significantly higher ANG expression as compared to controls with only PLGA/CNCs, recording 1.6-fold higher expression at day 3 and 2.8-fold higher expression by day 7. *In vivo* analyses showed that the pDNA-ANG nanocomposite-exposed rats had faster wound recovery and re-epithelialization [86].

CNCs have also been used as reinforcing agents in electrospun sheets, such as poly(3hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) [121] to increase their mechanical strength and hydrophilicity, allowing for long-term sustained release of cargos, such as tetracycline hydrochloride (TH). Similarly, PLA, a widely used nanofiber drug delivery

system, has been doped with CNCs. In fact, PLA is not a suitable material for drug delivery purposes considering its hydrophobicity and subsequent poor control of hydrophilic drug release; however, CNC has been able to overcome the unfavorable properties of PLA by imparting hydrophilicity to the electrospun nanofibers while enabling sustained control of drug release as a result of drug binding [84]. Polyethylene glycol (PEG) may often be selected as an additional compatibilizer to increase biocompatibility and further enhance the hydrophilicity through its hydroxyl groups. A typical process of preparing these fibrous platforms consists of mixing the PLA solution with CNC/PEG, followed by electrospinning.

Drug release levels and cytotoxicity are two key properties of composite fibers that must be assessed before animal studies and clinical trials. Compared to additive-free PLA nanocomposites, PLA/CNC/PEG provides a two-step release profile, depending on the amount of loaded drug. When a low concertation of drug is loaded (e.g., TH concentration ~ 3%), almost all of it will be released within a few hours. On the other hand, the nanocomposites loaded with ~ 10% TH provide ~ 60% burst drug release by the first hour followed by ~ 80%–95% release after ~ 43 days. Interestingly, a biodegradability assay revealed that the drug accelerated the scaffold degradation as a result of increased hydrophilicity [84]. These drug-eluting systems have been cytocompatible against MG-63 cells (human osteosarcoma).

Hybrid spherical CNC-colloidal titania patches for the sustained delivery of antibacterial and antifungal agents, such as triclosan [122], CNC-alginate films for ampicillin delivery [123], and gold-CNC-doped guar gum and poly(vinyl alcohol) (PVA) for transdermal diltiazem hydrochloride administration [124] are among other examples of CNC-based cargo delivery films. Interestingly, the controlled release of carvacrol and Cur from TEMPO-oxidized CNC-cyclodextrin films may hold promise for antibacterial food packaging [125].

Biodegradable polymers have offered new platforms for administering proteins and other biological macromolecules that demand additional structural support. PLGA has widely been tested as a delivery polymer with tunable degradability and mechanical properties to control the administration rate of biomacromolecules. PVA, a commonly used water-soluble and biodegradable polymer, is molecularly large enough to perform as a matrix for therapeutic cargos. Nanocomposites of PVA and CNCs have a higher Young's modulus than PVA alone, providing mechanically-robust structures [81]. PVA films doped with CNC have acquired up to 195% increase in the elongation at break at only 1% CNC. Besides mechanical reinforcement, thermal stability may also be improved by CNCs. Thermogravimetric analysis (TGA) of the polymer films has confirmed that the CNC nanocomposites have higher thermal stability than CNC-free PVA [81]. Some examples of common methods to make CNC-based cargo delivery systems are presented in Figure 6.

3. Bacterial cellulose (BC) and its composites for cargo delivery

Bacterial cellulose and nanocellulose (BNC) are crystalline biopolymers synthesized by specific types of bacteria, such as *Gluconacetobacter xylinus* [105,106]. Colloidal BNC, prepared from BC are typically negatively charged, which in concentrated BNC dispersions, form physical hydrogels with decent structural integrity, biocompatibility, and water binding

capacity, providing a desirable microenvironment for selective cargo delivery [130,131]. Biocompatibility, purity, and malleability of BC make it suitable for biomedical applications [131], such as topical treatment, as it can fit the epidermal surfaces and deliver antimicrobial or dermal therapeutics. Topical products typically do not require strict sterilization processes; however, challenges associated with emulsion stability, e.g., in cream manufacturing, require careful process design. Additive selection to engineer the physical properties (e.g., rheology) of nanocellulose-based formulations is of utmost translational importance. The topical products may be in the forms of creams, sprays, drops, and patches. Biocompatibility assays of topical BC patches over 24 h have resulted in clinical scores of zero, indicating no major health risks [132]. In the past decade, BC has mostly been used for wound dressing [133].

BC demonstrates desirable properties, including high tensile strength, crystallinity, and structural integrity, which are attributed to the intramolecular interactions of the hydroxyl groups on the BC surfaces [134]. BC has been integrated in various forms of medical treatment, including wound dressings, hydrogel-based oral drug delivery, and cargo scaffolds. Table 2 and Figure 7 summarize some of the most recent cargo delivery systems based on BC.

3.1. BC for cargo delivery

One of the challenges in designing drug delivery systems is to control the exposure location and delivery time [148]. Engineering the spatiotemporal release profile of cargos may reduce side effects and enable targeted clinical treatments [149]. BC has been studied as an infrastructure to warrant controlled drug delivery, which benefits from stability, biocompatibility, and a large surface area decorated with hydroxyl groups that allow for attaching bioactive components. BCs attain various desirable properties that are comparable to CNCs without plant-derivative byproducts (impurities), including hemicellulose, lignin, and pectin (P) [150]. While BNCs do not possess any antimicrobial or anti-inflammatory characteristics, upon functionalization and drug loading, they may be an excellent platform for wound dressing.

Sustained release of lidocaine hydrochloride, a drug typically used for numbing, and ibuprofen (IBU, a nonsteroidal anti-inflammatory drug) are among the examples of BC-mediated drug delivery [151,152]. BC may be modified with a glycerol plasticizer [153] to increase the flexibility and swelling ratio of scaffolds, while providing the sustained release of bioactive molecules, such as caffeine for cellulite treatment. Glycerol have safely been added to the BC to soften the treated regions without any adverse effect on the biocompatibility. Another interesting cargo for BC is bromelain, a protein derived from pineapple fruit, leaves, and stem, which is a proteolytic enzyme that has antibacterial capacity for treating diabetic ulcers [154], rheumatoid arthritis [155], angina pectoris [156], and bronchitis [157]. BNC loaded with bromelain has been used for wound healing [140]. The antibacterial and antioxidant activities of bromelain-loaded BNC biomaterials attest to their applicability as a drug delivery system for treating common pathogens. Loading the cargo in the BNC hydrogels typically demands the incubation of BNC membrane in the drug (e.g., bromelain) solution for 4 to 24 h (e.g., absorbing 7.94 mg/mL of the protein).

Hydrogels immersed in bromelain for 4, 8, 12, and 24 h showed identical enzymatic activity. The antibacterial properties of bromelain-attached CNC against *Staphylococcus aureus*, *E. coli* and *P. aeruginosa.*, characterized by the agar plate method, showed that all treated colonies were eradicated. Released bromelain solution enzymatic activity was higher than initial and residual enzymatic activity, and the total protein concentration in the released solution was the lowest. The BNC successfully adsorbed and delivered the proteolytic enzyme bromelain, which exhibited high enzymatic and bactericidal activities towards gram-negative and grampositive bacteria [140].

Current BNC wound dressing technology relies on combinatorial treatments, encompassing regeneration and vascularization promoters as well as bactericidal agents. Povidone-iodine (PI)-loaded BNC wound dressing has been able to improve the currently available polyhexanide-(PHMB)-loaded BNC dressing technology [146]. Using BNC, extracted from *Komagataeibacter xylinus*, drug loading is readily achieved by the incubation of nanocellulose in PI or PHMB solutions. While the uptake of the PI and PHMB by BNC dressings only reached to ~ 12%, the amount of PI loaded in terms of drug mass per unit mass, surface area, and volume of the wound dressing for BNC were about 10 times higher than PHMB [146]. An initial burst drug release was observed in the first 8 h whereby the burst release for PHMB was significantly higher than that of PI, recording 67% and 46%, respectively, and the PI-loaded BNCs exhibited a more prolonged drug release than PHMB. PHMB-loaded BNCs recorded a cumulative drug release of approximately 85% after 24 h, which plateaued after 48 h. The cumulative PI release was at around 73%, which progressively increased. Drastic cytotoxicity differences were observed for the PI- and PHMB-loaded BNCs against HaCaT keratinocytes. While both PI and PHMB exhibited some degrees of cytotoxicity, the PI-loaded BNCs exhibited significantly lower toxicity, observed at concentrations ~ 15 and 20 mg/mL, whereas a pronounced toxicity was measured at 2 mg/mL of PHMB-loaded BNCs. Antimicrobial activities against *S. aureus* showed a log-reduction greater than 7. These tests allowed for the quantification of half maximal lethal concentrations (LC) and half maximal inhibitory concentrations (IC), whereby the LC50/IC50 of PI-loaded BNC was 4.8 while PHMB-loaded BNC recorded a ratio of 229 [146].

Recently-developed wound dressings are engineered to maintain moist conditions, attain high efficacy and selective antimicrobial effects, and promote rapid healing. In one of the attempts to design effective wound dressings, octenidine has been incorporated in BNC. Octenidine is an antiseptic drug with desirable characteristics, such as biocompatibility, skin compatibility, non-resistance-inducing bactericidal properties, and 24 h-long skin remnant. BNCs, prepared from *K. xylinus*, were submersed in an octenidine solution for loading [137]. An initial burst release for all BNC fleeces was desirable to achieve a therapeutically-relevant level. Equilibrium conditions were reached after 24 h, followed by a slow release. The biocompatibility of octenidine-loaded BNCs was tested against keratinocytes, a major cell type in human skin, indicating that no significant cytotoxicity was observed at 0.1–1% octenidine/BNC after 1, 24, and 48 h. However, octenidine/BNC ~ 10% drastically decreased the cell viability after 24 h. The antimicrobial activity against cultures of *S. aureus*, a common bacterial strain involved in wound infection, was also confirmed. Interestingly, the antimicrobial potency was preserved for BNC fleeces that were prepared

and used after 6 months of storage [137]. Importantly, antimicrobial activity against the *S. aureus* was more pronounced than the cytotoxicity against HaCaT keratinocytes. The octenidine-loaded BNCs may serve as a powerful wound dressing with practical commercial benefits, such as potency after prolonged shelf storage [137].

BC has also been used for the delivery of nucleic acids. Singhsa *et al.* [127] have prepared bacterial celluloses using three different bacterial strains of *K. xylinus,* followed by hydrolysis using three different acids: hydrochloric acid, sulfuric acid, and a mixture of both. These BCNCs were tested for cytotoxicity and efficacy for nucleic acid delivery post-cationic (amino) functionalization. The modified cationic BCNC was simply mixed with siRNA to yield BCNC-siRNA complexes. The BCNC-siRNA complex formation was assessed using agarose gel electrophoresis, which indicated that modified BCNCs with a net negative ζ-potential failed to completely form a complex with the siRNA, while those with a net positive ζ-potential successfully yielded full complexation with siRNA. These results attest to the promising potential of surface charge modified BCNCs as cargo delivery vehicles for nucleic acids and proteins [127].

3.2. BC composite hydrogels and membranes

Hydrogels made up of bacterial nanocellulose have emerged as potential platforms for targeted drug delivery with remarkable biocompatibility, structural stability, water retention, and customizability. BNC hydrogels can be fabricated using as low as 1% nanocelluloses, which may benefit form a large water content. Conventional methods for loading drugs in BNC hydrogels and other BNC-integrated matrices and scaffolds involve the immersion of the BNC delivery system in the drug solution. This allows for the drugs to be loaded via diffusion and adsorption; however, the downside of this process is the prolonged loading time, which is not a practical for clinical and industrial purposes. Composite BNC/poly(N-methacryloyl glycine) membranes have been incubated with diclofenac sodium salt (DCF) for 48 h, resulting in ~ 100% drug loading (5 mg cm^{-2}) and a pH-regulated controlled release with the highest release at the neutral pH [141]. An alternative method for drug loading involves vortexing for a few minutes, resulting in the high-speed loading of cargos, e.g., bovine serum albumin as a model protein (Figure 8a) [136]. Interestingly, the BNCs loaded through the conventional method showed a much faster BSA release as compared to the vortexing technique. Significant differences were observed after 24 h, and by 72 h, the model drug loaded with the conventional technique recorded a 91.2% release as opposed to a 62.4% release by the vortexing technique. Scanning electron microscopy (SEM) images revealed interesting outcomes that could serve as explanations for the observed drug release profile. As compared to the untreated BNC, the BNC treated with conventional drug loading methods exhibited a slight increase in fiber thickness and pore size. On the other hand, BNC treated with the vortex exhibited a significantly denser network of fibers with thicker fibers and partial pore closures [136]. Besides physical loading, drugs, such as amoxicillin may be chemically conjugated to BCs, e.g., to produce wound dressing sponges [158].

High swelling capacity is beneficial to load cargos in BCs. BC/acrylamide hydrogels may be prepared using microwave radiation under a basic condition (NaOH/urea), which demonstrate a higher theophylline (a drug for respiratory medical conditions) loading

capacity than BC-free polyacrylamide scaffolds, providing controlled release in 24 h [159]. While clinical drug delivery demands hydrated hydrogels, long-term storage of hydrated materials raises issues regarding storage and the risk of bacterial contamination. Air-drying hydrogels results in microstructure collapse, which may deteriorate their bioactivity [135]. Lyophilization, supercritical carbon dioxide, ethanol drying, or hot-pressing are all expensive, resource-dependent operations that result in low-quality BNC hydrogels. To preserve the desired BNC microstructure and functionality for drug delivery, the dehydration of crystalline hydrogels may be conducted using several supplements, encompassing glucose, sucrose, lactose, trehalose, sorbitol, mannitol, PEG, magnesium chloride salt, and sodium chloride salt [135].

Supplementing 10% (w/v) magnesium chloride ($MgCl_2$), glucose, and sucrose resulted in the highest re-swelling and drug delivery properties, which offered the best dehydration methods for preserving BNC structure and function [135]. Magnesium chloride supplement retained the largest amount of water after dehydration relative to never-dried BNC. After 168 h of rehydration, $MgCl_2$-supplemented BNC reswelled to ~93% of its initial volume before drying and ~ 88% of its original weight. Glucose-, sucrose-, and sorbitol-treated biomaterials reswelled to above ~ 60% of their original volumes. SEM images revealed that the top and bottom surfaces as well as the cross-section of glucose-, sucrose-, and magnesium chloride-supplemented biomaterials retained their original three-dimensional structure after dehydration as compared to air-dried biomaterials that suffered from fiber aggregation and structural failure [135]. Interestingly, glucose-supplemented BNC released ~ 52% of a model drug (azorubine) in 1 h and ~ 95% in 4 h, which has a better control of the cargo release than the air-dried BNC releasing ~ 98% of the drug in the first hour (Figure 8a). Accordingly, supplementing BNC with carbohydrates or inorganic salts may facilitate the storage and transportation of BNC while maintaining the nanocrystal structure and function [135].

Interestingly, the physicochemical properties of BC have been engineered by simple drying methods. The integration of BC into single-excipient based BC matrices undergoing various drying methods, such as freeze-drying and oven-drying before and/or after drug loading (famotidine or tizanidine) has been investigated [160]. Higher drug loading was obtained for hydrated and partially hydrated BC matrices as compared to the freeze-dried ones. Hydrophilicity and porous fibrous network facilitated drug diffusion through the matrix as opposed to the freeze-dried matrix, wherein lacking these properties resulted in noticeable fiber aggregation and reduced water retention capacity. BC matrices loaded with famotidine or tizanidine had more than 80% burst drug release in the first 15 min in the simulated gastric fluid.

3.3. BC composite films

One of the main motivations to prepare BC films is their applications in wound healing, wherein functionalized substrates are required to remain on the lesion and provide healing and antibacterial effects. Traditionally, burn wounds have been covered with gauze and cotton wool; however, they adhere to the wound and are difficult to remove. Accordingly, alternative biomaterials, such as collagen, chitosan, and alginates have emerged for wound

dressing [161,162]. The focus of research has been on the wound dressing biomaterials with drug releasing properties that leverage the healing process [163,164]. While various types of transdermal patches have been developed for the strategic and controlled delivery of drugs, increased microbial resistance has imposed unmet challenges to effective wound healing and wound infection prevention. To address this issue, novel approaches have been explored to achieve the dual release of proteins and antibiotics, leveraging bio-functionalities against antimicrobial resistance [165–167].

In a recent effort, the dual release of proteins and antibiotics from hybrid BC films has been investigated. BC, extracted from *Komagataeibacter hansenii*, was mixed with high methoxylated pectin (HMP) and modified with HSA and levofloxacin (Levo, an antibiotic agent). BC-HMP provided a lower burst drug release, enabling the controlled release of both Levo and HSA as compared to the unmodified BC. Cytotoxicity studies have suggested that this dual drug release system is biocompatible as Levo toxicity was attenuated upon loading in HMP, recording ~ 90% cell viability at 200 μg/mL (as compared to ~ 71% for free Levo) and ~ 100% cell viability at 100 μg/mL (as compared to ~ 89% for free Levo) [168]. Importantly, Levo-HSA-HMP had an antimicrobial activity against *S. aureus* and demonstrated promising outcomes not only for infection prevention but also for enhanced cell proliferation and tissue regeneration [168].

Another antimicrobial agent that can be delivered using BNC films is fusidic acid (FuA). FuA is a commonly used bactericidal drug, which is effective against drug-resistant bacteria. FuA can be loaded in BNC films by immersing the films in a sodium fusidate solution. The antibacterial effect of FuA-loaded BNC against *S. aureus*, tested using the disk diffusion method wherein the biomaterials were placed on *S. aureus* lawns on an agar plate and examined for the degree of clearance of the lawn surrounding the composite [169], demonstrated that the BNC films loaded with 0.4 g/L of FuA resulted in a 34 mm-diameter inhibition zone. Recorded inhibition zone diameters were 27, 30, and 32 mm for loading concentrations ~ 0.1, 0.2, and 0.3 g/L, respectively, showing the high potency of FuA-doped BNC nanocomposite films as a bactericide wound dressing.

PVA-BC hydrogels have been produced in monolayer and multilayer forms to load and release vanillin as an antimicrobial agent in a controlled manner that is regulated by the film composition [170]. Huang *et al.,* [171] compared the release of berberine (a plant-based quaternary ammonium salt) from BC in different simulated fluids and found that the slowest berberine release occurred in simulated gastric fluid (SGF), containing sulfuric acid, and the highest release rate took place in the simulated intestinal fluid (SIF). SEM imaging confirmed that BC nanofibers became thicker in acidic solutions, resulting in smaller pore sizes that restricted the cargo diffusion rate.

Alternatively, BC may be impregnated with antimicrobial zinc oxide (ZnO) nanoparticles (Figure 8b, left panel) [172] or silver nanoparticles [173] for wound dressing. The BC-ZnO nanocomposites can be synthesized via the incubation of BC sheets in a ZnO nanoparticle dispersion [142]. These nanocomposites have antimicrobial activity against *E Coli*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, the first three of which are Gram negative bacteria and the last Gram positive. Treatment with BC-ZnO

showed a zone of inhibition that was similar in diameter to that of silver sulfadiazine (SD), a standard antimicrobial drug. Percent inhibition was calculated by dividing the BC-ZnO zone of inhibition by that of SD, all of which had approximately 90% inhibition for all four bacterial species. The effectiveness of wound healing and antibacterial activity were also confirmed in the *in vivo* tests conducted on burn mouse models. The average wound area was observed over 0, 5, 10, and 15 days, in which the BC-ZnO always showed significant wound healing effects (p   0.001) [142]. In fact, the recorded average wound area for the BC-ZnO-treated mice (269.3, 195.6, and 98.3 mm^2 for 5, 10, and 15 days, respectively) was comparable to those of the SD-treated mice (287, 148.3, and 66.6 mm^2, for 5, 10, and 15 days, respectively), supporting the potency of BC-ZnO nanocomposites.

BC may be combined with synthetic nanomaterials to overcome their limitations. BC/ graphene oxide (GO) nanocomposites overcome the non-specific binding of GO to proteins in biological systems. BC/GO nanocomposites can readily be prepared *in situ* by adding GO to the culture media of cellulose-producing bacteria (e.g., *Komagataibacter xylinus* X-2) [139], providing a higher capacity for loading drugs than neat BC. Despite increasing the surface area and improving the loading capacity, added GO may have adverse effects on cell viability. As an example, 0.48% GO was not suitable for drug delivery as it demonstrated inhibitory or cytotoxic effects. Ibuprofen delivery using BC/GO and GO-free BC was tested at near neutral pH (~ 7.4) and acidic pH (~ 1.2) at the physiological temperature (37.0 ± 0.5°C), which showed a non-Fickian diffusion-regulated release and a zero-order transport rate, respectively. For both acidic and near-neutral pH, BC/GO released IBU at a more controlled manner than neat BC over 24 h [139].

To increase the hydration capacity of BC, dissolved cellulose additives, such as carboxymethyl cellulose (CMC), have been used without significantly compromising the crystallinity and thermal stability of BC. Modifying BC with polyanions has enabled the regulation of physical and chemical interactions with target molecules, e.g., water and drugs, to design drug delivery systems with desirable swelling, loading, and release rates [174–178]. BC can be modified with solubilized celluloses *in situ*, for example, via adding 1% CMC to the bacteria culture medium (Figure 8b, right panel) [143]. Increasing the degree of carboxylic acid substitution (DS) of CMC had a positive effect on hydration capacity and swelling ratio; however, at high concentrations, the crystallinity index and storage modulus of BC composites decreased. BC/CMC (DS ~ 0.7) swelled more than neat BC, BC/CMC (DS ~ 0.9), and BC/CMC (DS ~ 1.2); however, the liquid intake for neat BC was 490%, and BC/CNC composites with DS ~ 0.7, 0.9, and 1.2 absorbed 958%, 86%, and 578% water, respectively [143]. Interestingly, the high porosity of BC/CMC (DS ~ 0.7) resulted in the highest swelling ratio. Hydroxyl-ester interactions in BC/CMC (DS ~ 0.9) composites increased the matrix stiffness, which decreased the liquid absorption and the swelling capacity. In all samples, 70–80% MTX was released in the first 15 min, followed by a steady drug release thereafter. BC/CMC (DS ~ 0.9) provided the lowest MTX release rate, yielding 80% release after 180 min, whereas BC/CMC (DS ~ 0.7) released 96% of MTX over the similar period [143].

4. Cellulose nanofibrils (CNF) for cargo delivery

Biomedical research on CNFs has mainly revolved around studying the mechanical, chemical, thermal, and simulated loading/release properties of the nanocellulose composites. Recently, immense attention has been drawn towards biobased materials to design and produce a broad range of environmentally-benign materials with added values. The abundancy of plant biomass and the tunable mechanical and rheological properties of CNF have rendered this nanocellulose an attractive additive for a variety of polymer nanocomposites [117,179]. Different forms of CNF-based cargo delivery systems, encompassing colloidal particles, hydrogels, shells/capsules, aerogels/foams, and films are summarized in Table 3 and Figure 9.

4.1. CNF for drug delivery

Formulation and development of drug delivery systems have faced several challenges in the past decade. Poor aqueous solubility of cargos remains a common obstacle in developing effective delivery systems [196,197]. To overcome the limitations of sparingly soluble drugs, CNFs have recently been noticed in the pharmaceutical industry [181,198]. Unmodified CNFs are amphiphilic nanocelluloses decorated with hydrophilic and hydrophobic surfaces, which are able to adsorb hydrophobic drug molecules and nanoparticles and increase their colloidal stability. In addition, the nanocellulose-mediated mechanical reinforcement of drug carriers, such as starch, may leverage the controlled release of active molecules [199]. These properties have been verified by multiple studies confirming the favorable molecular interactions between CNF and poorly-soluble drugs [181]. Small poorly-soluble surfactant-like drug molecules can be adsorbed by CNF and change the surface energy of nanocellulose. This can provide the Pickering stabilization of surfactant-free nanocellulose-based emulsions [200]. CNF, produced as microparticles with tight fibrillar network, has been loaded with drugs via spray drying. This strategy has resulted in the sustained release of drugs for several months, regulated by the drug solubility.

Capsules with versatile core functionality and tunable mechanical stability have been explored for potential applications in drug delivery [201,202]. Fiber-based reinforcement is inspired by the nature's architectural solutions for building mechanically resilient constructs [203]. Mechanically-robust CNF capsules have been prepared using the layer-by-layer (LbL) technique (Figure 10) to fabricate small-scale scaffolds with on/off permeability regulated by salinity (ionic strength) for the on-demand loading/unloading of cargos, such as model fluorescent-labelled dextran [187,188]. This platform also holds promise in delivering drugs in a time-dependent manner. The modulus of wood-derived CNF is in the order of 150 GPa [204], supporting the mechanical integrity of CNF nanocomposites, such as capsules. Tunable oil core of CNF-based capsules may successfully contribute to the delivery of poorly-soluble drugs (Figure 10) [188,205].

Although CNF is a commercially available nanomaterial with potential applications in food industry, e.g., for decreasing the intestinal digestion and absorption of fat [206], a few studies have been conducted on its safety and the potential hazards post-accidental exposure [207,208]. Biological reactions through inhalation and dermal contact caused by wood-derived carboxymethylated or cationic CNF have been simulated *in vitro* [209]. Antibody

assay (AB) and lactate dehydrogenase (LDH) assays show that CNFs do not have adverse effects on the metabolic activity of immune, dermal, and lung cells. THP-1 cells, as an *in vitro* cell model for immune modulation, had a significantly high metabolic activity when treated with CNF. When cell culture media were incubated with unmodified CNF, a significant increase in the pro-inflammatory cytokines, such as TNF-α and IL1-β, was observed; however, the chemically-modified CNFs did not significantly affect the cytokine levels. Furthermore, CNFs did not result in a significant increase in reactive oxygen species levels, suggesting that the oxidative potential of the cells was not affected by the CNFs. Transmission electron microscopy of CNF-exposed cells indicated no significant change in the cell shape and no CNF uptake by macrophages [210].

4.2. CNF composite hydrogels

Naturally-derived hydrogels have been gaining popularity; these hydrophilic polymer networks with widespread applications across multiple fields benefit from biocompatibility, easily adjustable chemical and physical properties, and high water contents [211–213]. Hydrogels are commonly used as drug delivery systems. In a simple approach, hydrogels undergo swelling inside a drug suspension to capture the drug and subsequently release it with programmed temporal and/or spatial precision. To this end, CNFs may form the backbone of hydrogels or be used as an additive. Common CNF-based hydrogels have been reviewed in literature [48].

CNF may be physically self-crosslinked, e.g., using two oppositely-charged CNF batches [214], or be incorporated in a wide range of polymeric matrices to increase their mechanical integrity, hydrophilicity, and thermal stability, and control the cargo loading and release rates. A broad range of polymers, including PVA, a biocompatible and non-toxic polymer, may be blended well with CNF to form reinforced hydrogels. These hybrid hydrogels may have applications in delivering chemotherapeutic compounds, such as cisplatin, an antineoplastic drug to treat tumors [182]. The CNF-PVA hydrogels attain a significantly high swelling ratio, exhibit a tunable drug release profile (e.g., from 0.5 h to 7 h at 1 wt% CNF), and are able to reduce the side effects of cisplatin, such as nephrotoxicity. Increasing the pH (from 1.2 to 7.4) results in an increased cumulative drug release, indicating that the slightly basic solution found in the small intestine is an ideal environment for maximum drug release.

Several types of cancers, such as melanoma (skin cancer), have undergone increased resistance against chemotherapeutic drugs, such as Dox, demanding localized chemotherapy to increase the drug concentration at the tumor site [215–219]. One solution under investigation is the use of, often electrospun, nanofibers to benefit from their similarity to the fibrils (e.g., collagen) in the extracellular matrix (ECM). Nanofibrous cellulosic biomaterials may enhance interfacial cell-matrix interactions, perform as a physical barrier to prevent tumor cell invasion, and provide a high-capacity drug depot [220,221]. Cellulose nanofibers may also provide injectable nanofibrous biocompatible biomaterials decorated with hydroxyl and carboxylic acid groups that undergo electrostatic or hydrogen bonding with drugs [191]. The adsorption of Dox to the surface of CNFs increases the localized

cytotoxicity against the cancer cells compared to free Dox, which decreases the required drug dose for chemotherapy.

The ECM degradation and remodeling are crucial factors in inhibiting the migration and metastasis of cancer cells [222–225]. Novel treatments to reduce the risk of metastasis are highly demanded for treating aggressive malignant cancers, such as melanoma. The properties of nanofibers such as high surface area, high drug loading capacity, sustained drug release, and similarity to the ECM may offer effective treatments to reduce the risk of metastasis by promoting the adhesion of cancer cells to the barrier biomaterial and blocking the cell migration [183]. Metformin, an anti-cancer and anti-metastatic drug, is more effective if delivered directly to the tumor site. This drug is positively charged at physiological pH, enabling it to electrostatically adsorb to negatively-charged CNF. The amount of drug attached to the nanofiber surface is independent of the drug concentration and can be manipulated by changing pH to protonate/deprotonate the carboxylic acid groups of CNF. The rheological properties of these gels are not significantly affected by the drug loading, providing an injectable drug delivery platform with suitable injection forces for minimally-invasive clinical procedures [183]. An MTT assay showed that loading CNF with 17.6 mg/mL metformin was able to partially inhibit the growth of melanoma cancer cells without affecting non-cancerous cells. Cell attachment occurred in the drug-modified cellulose nanofiber (Met-CNF) gel for nanofibers with a diameter greater than 400 nm. Drug-eluting biomaterials that promote cell adhesion may hold promise in preventing cell migration [183].

Natural hydrogels, such as those consisting of deacetylated α-chitin nanofibers may be combined with drug (e.g., 5-FU)-loaded negatively charged TEMPO-oxidized cellulose nanofibrils (TOCNF). TOCNF retains the original crystallinity of CNF and disperses in water [226]. α-chitin fibrils have a high aspect ratio, strong mechanical properties, and excellent biocompatibility and degradability [227,228]. 5-FU is an anticancer drug with low solubility, which induces multiple undesirable side effects when intravenously injected or infused. Thus, administering this chemotherapeutic drug orally may help minimize the side effects [189]. Both TOCNF and α-chitin not only reinforce the structure of carriers but also impart a slow drug release profile. When mixed, physical crosslinking occurred due to the electrostatic interactions between the anionic TOCNF and cationic chitin. The lowest ζ-potential of these polyplexes was found in a hydrogel containing 40% partially deacetylated α-chitin nanofibers, which indirectly indicates that this composition has the highest crosslinking density. Increasing the chitin content beyond the optimum value increases equilibrium swelling ratio as a result of the free amine and hydroxyl groups undergoing hydrogen bonding, which promote water absorption in the nanofiber networks. The hydrogel containing 40% chitin had the lowest surface charge and provided the highest 5-FU loading capacity and efficiency (~53%) in PBS at 37 °C. When the ratio was changed, the hydrogel loading capacity along with drug release percentage declined [189].

An emerging application of CNF is probiotics delivery [190]. A composite macrogel made up of cellulose fibers and nanofibers functionalized with carboxyl groups (Figure 11a, b) performed as a suitable platform to deliver probiotics to a specific region of small intestine. To encapsulate and protect the probiotics from early deactivation by gastric fluids,

carboxylic groups on the cellulose surface are favorable, imparting pH-responsive properties to the nanocellulose carrier. The carboxyl groups on CNF, typically yielded by TEMPO-mediated oxidation, regulate the pore size of hydrogel carriers, rendering them suitable for the controlled release of probiotics (Figure 11c). CNF macrogels exposed to SIF and SGF exhibited tunable swelling behavior and pH responsiveness. Increasing the number of carboxyl groups on the nanofibers resulted in a slight shrinkage in SGF and increased swelling when transferred to SIF. Furthermore, the carboxyl groups performed as a pore size regulator while the cellulose backbone strengthened the porous structure. The maximum release of viable *L. plantarum* (model probiotic) from the carrier was more than 2.23×10^7 CFU/mL (Figure 11d) [190]. Porosity and the pore size of macrogels leveraged the loading and amplified the colonization of *L. plantarum* cells. CNF gels prepared from dietary fibers with selective permeability may be an excellent prospect for targeted delivery in the gastrointestinal tract.

In addition to drug delivery, CNF composite hydrogels have been used as hemostatic wound dressing materials [229–231]. Calcium-mediated crosslinked anionic CNF, kaolin-CNF, and collagen-CNF composite hydrogels have been explored as potential hemostatic wound dressing biomaterials. CNF biocompatibility and ability to induce "good" hemostasis, defined as the rapid initiation of the coagulation cascade, platelet adhesion, and complement system activation, have been highlighted [184]. Kaolin, an aluminum silicate, was chosen as a hemostatic agent to activate the intrinsic pathway of coagulation. Collagen, commonly used as a hemostatic agent, contributed to the initial activation of platelets leading to hemostasis and promoting later phases of wound healing. TEMPO-mediated oxidation yielded highly charged CNFs, which were physically crosslinked with calcium ions to produce self-standing hydrogels.

CNF hydrogels resulted in C3a and sC5b-9 activation; however, the amounts were labeled as non-significant compared to the commercial dressing (AQUACEL® Extra), showing that the gels were not able to promote complement system activation [184]. However, the low complement activation by CNF hydrogels were beneficial for chronic wound healing and the treatment of early-stage wounds. Coagulation cascade was studied by incubating the hydrogels in whole blood, followed by measuring the platelet number and the level of thrombin-antithrombin (TAT), which showed significant reduction in platelet number and considerable TAT formation compared to the control (AQUACEL® Extra). The CNF-kaolin hydrogel displayed the most efficient coagulation activation through platelet reduction and TAT formation. All three hydrogels in comparison to the control showed greater thrombin peak height and faster time-to-thrombin-peak, indicating the endogenous thrombin potential correlated with a fast coagulation [184]. The hydrogels displayed an accelerated onset of thrombin formation compared to untreated platelet-free plasma (PFP) and AQUACEL® Extra. Thrombin and factor XII formation and platelet reduction attested to the potential of CNF-based hydrogels for effective blood coagulation and rapid clot formation. These biomaterials may be used for advance therapeutic wound dressing.

CNF has also been used to decrease the cytotoxicity of polymers, such as chitosan. The addition of CNF may help decrease the gel formation time, increase porosity, and improve the biocompatibility of hydrogels both *in vitro* and *in vivo* [232]. Natural thermosensitive

injectable hydrogels can be prepared for minimally-invasive procedures using TOCNF and chitosan/glycerophosphate (CS/GP). A high GP content have cytotoxic effects on cells, however, adding TOCNF to the CS/GP hydrogels enhanced the proliferation and adhesion of fibroblast (L929) and pre-osteoblast (MC3T3-E1) cells. Subcutaneous injections *in vivo* showed that these hydrogels led to an inflammatory response in rats. After 14 days, tissue remodeling was evident with an increased M2 type macrophages, which indicated the commencement of wound healing. The hydrogels containing TOCNF accelerated wound healing by inducing a pro-inflammatory response after seven days of implantation [232].

4.3. CNF composite films

CNF films are mainly fabricated by spinning [233], filtration/drying [234], spray coating [235,236], electrospinning [237], and layer-by-layer assembly techniques [235]. Electrospinning produces nanofibrous scaffolds from a polymer base, which attain nanoporous structures, flexibility, and a high surface area-to-volume ratio, all of which are suitable for drug delivery purposes. CNF films may entrap poorly-soluble drugs and provide enhanced drug loading and sustained release for medical conditions such as inflammation and cancer. An important example is the inflammatory bowel diseases [237], demanding colon-specific drug delivery to overcome the challenges associated with the reduced proteolytic activity. In this case, an extended residence time is a favorable property for the release and absorption of drugs and proteins.

Film coating used for colonic drug delivery relies on colonic enzyme-degradable biomaterials, such as resistant starch (RS) produced by the hydrothermal modification of starch [238], which often lacks structural integrity. Polymer composites are commonly used to leverage the performance of film-based cargo delivery agents. Pectin is a biodegradable, biocompatible, and nontoxic heteropolysaccharide that protects the films from amylase- and protease-induced digestion [239]. Nanocelluloses, such as CNF and BNC, are a viable solution to leverage the performance of RS/P nanocomposite films. Macroscopic evaluation of CNF and BNC-RS/P nanocomposites showed that the films with a higher cellulose nanofiber concentration were thicker [180]. Field emission gun SEM indicated that the BNC covered the surface of the samples, while the CNF films showed more homogenous mixing. However, CNF was more effective than BNC in increasing the elongation without breaking, yielding elongation at break ~ 5.4 to 9.2 times higher than the control [180]. These improved properties are likely a result of the hydrogen bonds between the film and the crystalline/amorphous regions of the nanocelluloses. Additionally, water vapor permeability (WVP) measurements showed that the films provided effective water protection with 3.9–5.8 times reduced permeability due to the nanocellulose-filled pores [180]. The incorporation of nanocelluloses in the films may also contribute to the sustained release of drugs. For example, MTX had the highest release from RS/P control film and the lowest from the RS/P-0.5CNF, verifying that the CNF and BNC films with reduced porosity yielded prolonged drug release [180]. These findings suggest that nanocellulose-reinforced RS/P films may be a cost-efficient and viable oral drug delivery system for insoluble drugs to treat bowel-inflammatory diseases. Furthermore, the transdermal delivery of drugs, such as Ketorolac tromethamine (KT, a non-steroidal analgesic) using CNF-based nanocomposite films (e.g., with chitosan) has alternatively been explored [240].

Ion-responsive drug delivery platforms, such as microcapsules, have been fabricated from composites of CNF with hemicellulose (xyloglucan) and pectin. These polysaccharides are typically non-immunogenic, non-toxic, biocompatible, and often biodegradable. Ion-mediated changes in permeability enables these microcapsules to perform as on/off switches, which may be engineered by tuning the doping concentration of CNF and hemicellulose. For example, increasing hemicellulose content decreases the ion sensitivity of CNF-based systems [187]. To overcome the poor binding of hemicellulose and pectin (sourced from apple, AP) to CNF, multilayer CNF/Xyloglucan-amyloid (XyG)/AP with various compositions and layers have been coated on sacrificial spherical calcium carbonate microparticles. To decrease the AP/XyG incompatibility, pectin was used to increase the stability of microcapsule walls. In water, capsule walls swell and absorb dextran (e.g., 20 kDa and 70 kDa) [187,188]. The pore size and permeability may be controlled using salts; for example, a NaCl solution decreases the permeability as a result of pore size shrinkage.

CNF films provide a prolonged, sustained release (e.g., up to several months) of poorly water-soluble drugs, such as beclomethasone dipropionate, indomethacin, and itraconazole [234]. Typically, a CNF suspension is mixed with poorly water-soluble drugs, followed by filtration to collect the layered CNF network embedding the undissolved drugs. Next, CNF undergoes hornification (irreversible inter-fiber binding), forming a drug-loaded film. Compared to spray drying, this approach provides higher drug loading and entrapment efficacies (> 90%). Interestingly, the films attain excellent mechanical properties and readily hold a desirable shape [234]. The immediate release of drugs, such as indomethacin, from CNF nanopapers is also plausible. Engineering the fiber structures, a short release time (~ 10–20 min) has been achieved [241]. This has been attributed to the CNF-mediated drug solubility improvement, large pores, and the low tortuosity of the composites.

CNF nanocomposites for drug delivery may be fabricated using inorganic nanoparticles, such as titanium oxide [242] and magnetite. Magnetite (Fe_3O_4)-silver(I) oxide (Ag_2O) quantum dots/cellulose fiber nanocomposites have been fabricated to deliver a chemotherapeutic cocktail of etoposide and MTX for lung, breast, and skin cancer [185]. The drugs were dissolved in water and added to the aqueous dispersion of Fe_3O_4-Ag_2O quantum dots/cellulose fibers, followed by drying. The release of etoposide and MTX was then evaluated at physiological pH. Etoposide was released at a constant rate, recording ~ 79% in the buffer after 24 h, and the MTX was released at a prolonged constant rate, requiring a longer incubation time to reach equilibrium: ~ 64% release after 30 h [185]. The different release kinetics of drugs may be explained by the difference in the solubilities: etoposide is more soluble than methotrexate and can readily be released. This nanocomposite was a safe drug delivery system that targeted melanoma cancer cells.

Solubilized celluloses, such as CMC and hydroxypropyl cellulose (HPC), are an important class of celluloses, which play a key role in developing nanocellulose films [243]. As an example, for the delivery of sulfisoxazole (SFS), a weakly acidic and insoluble sulfonamide antibacterial drug, HPC has been added to the inclusion complex (IC) of cyclodextrin (CD), a non-covalent complex that binds the poorly soluble drug to the hydrophobic cavity of the water-soluble CD structure [185]. The release profile was biphasic in which an initial fast release followed by a slower, constant release over 12 h were achieved. Because of the large

surface area of nanofibers, the total amount of SFS released from the HPC/SFS/HPβCD-IC-nanofibers was significantly greater than the amount released from both HPC/SFS/HPβCD-IC films and SFS powder samples. Furthermore, the HPC/SFS/HPβCD-IC-nanofibers initially released SFS slower than the nanofiber-free film [185].

4.4. CNF aerogels/foams

CNF aerogels/foams (also known as nanofoams) provide sustainable drug delivery platforms with positive buoyancy [244,245]. Drugs, often demonstrating poor solubility in aqueous media with a short absorption window, may be readily loaded in CNF aerogels. The rheological, barrier, and physicochemical properties, readily formable structures (particles, capsules), and the ability to stabilize oil/water and air/water interfaces along with large surface area-to-volume ratios enable CNF to perform as a decent excipient of poorly water-soluble drugs [246]. This can induce a tunable and prolonged residence time of drugs and improve their bioavailability. Dry CNF-based aerogels/foams are macroporous materials, fabricated from hydrogels in which the liquid has been replaced by gas (air) [48]. Typically, the hydrogel undergoes freezing, solvent-exchange, or foaming in the presence of surfactants, followed by the direct removal of frozen solvent, e.g., through sublimation. The release profile may be controlled by tailoring the physicochemical interactions between the cellulose matrix and nanoparticles. Different sources of CNF have different release capability, which may be used to engineer aerogels for immediate or sustained release.

Wet foams may be prepared using cationic CNF and lauric acid sodium salt (surfactant) to bind drugs, such as riboflavin, which can be followed by drying to obtain dry foams [247]. This platform absorbs drugs in its flexible structure that may be molded/cut into different shapes and sizes. Maintaining drug loading capacity and carrier weight constant, the drug release kinetics may be modified by changing the foam size. For example, after 24 h, an 8 mm thick rectangular foam released ~ 59% of the drug, whereas a 0.6 mm thick foam was able to release 78% of the drug in 8 h. The release rate may be accelerated by using a thinner foam: a 0.009 mm thick foam with the same outer surface area and total mass released ~ 72% of the drug within 1 h. CNF aerogels have been explored as gastroretentive drug delivery vehicles [186]. Important properties of CNF aerogels that need to be characterized for optimal drug delivery encompass surface morphology, swelling ratio at different pH, floating behavior (the time that the material remains buoyant), tensile strength, mucoadhesion, and pH-dependent drug release.

Cargos are typically loaded in the aerogels by physical absorption/adsorption, which is directly correlated to the fiber concentration: the higher the fiber content, the higher the loading capacity. The swelling ratio of CNF aerogels in physiological media depends on the nanocellulose charge content. As an example, a swelling ratio ~ 200% at physiological pH has been recorded after 24 h, which was affected by the accessibility of hydrophilic sites [186]. The drug-loaded CNF may remain floated for several hours due to the air entrapment in the aerogel. Drug loading may decrease the mucoadhesive strength of the aerogels: drug-loaded CNF aerogels had an adhesion strength ~ 18.19 ± 3.64 g/cm^2, which was lower than the CNF aerogels (36.9 ± 3.8 g/cm^2). This was attributed to the decrease in the surface area of drug-loaded CNF [186]. Similarly, tensile strength was lower in drug-loaded CNF. After 24

h, ~ 69% of the drug was released at pH ~ 1.2, whereas ~ 78% was released at pH ~ 7.4. A pharmacokinetic study showed that the aerogels were able to improve the oral bioavailability of drug by controlling the release rate [186]. The high surface area, unique swelling tendency, and hygroscopic nature of CNF enhance physical drug loading and controlled release. The excellent floating capacity and suitable mucoadhesion renders CNF aerogels an ideal candidate for gastroretentive drug delivery. Some examples of CNF-based cargo delivery platforms are summarized in Figure 12.

5. Translational aspects of nanocellulose-based cargo delivery

Despite numerous *in vitro* and a few *in vivo* studies focused on characterizing nanoengineered celluloses crystals and fibers for cargo delivery applications, translating these classes of nanomaterials from bench to bedside is contingent upon (i) gaining a thorough understanding of the physical and biological challenges associated with *in vivo* colloidal stability, targeting, permeability and uptake, cytotoxicity, hemocompatibility, and bioaccumulation, (ii) large-scale production, processing, analysis, sterilization, and quality control, (iii) cost optimization and competitiveness against current technologies, and (iv) regulatory criteria. Nanocelluloses are produced in a variety of morphologies, sizes, crystal structures and content, and surface chemistry [249] each of which may have a unique *in vivo* behavior. Provided the high cost of animal studies and clinical trials, the choice of a base nanocellulose platform that meets the translational criteria is pivotal for the successful commercialization of this technology. Besides the comprehensive *in vivo* evaluation of commercially available nanocelluloses, their production must be revisited to enable good manufacturing practice (GMP), followed by a cost estimation for characterizations [250], such as handling, drying, sterilization, redispersion, surface charge determination, elemental analysis, and imaging.

Safety and life cycle are other important translational aspects nanocellulose-based delivery technologies. Cellulose is typically not absorbed systemically following oral administration, therefore it may have insignificant toxic potential, which renders it a generally-recognized-as-safe (GRAS)-listed material for oral use. GRAS designation may not apply to parenteral or pulmonary drug products. Pulmonary toxicity must be thoroughly assessed as a risk for instilled/inspired/inhaled nanocellulosic materials in diverse forms [208,251–255]. Despite some reports on the dose-dependent toxicity of nanocelluloses, this class of nanomaterials are mainly distinguished as non-cytotoxic under regulated dosages. As an example, bacterial cellulose has been identified non-irritating and biocompatible, which does not trigger a harsh inflammatory reaction and has a longstanding history of safe human consumption in Pacific countries [256]. However, according to the ISO-10993 standard for Biological Evaluation of Medical Devices [257], the extractables/leachables of nanocelluloses must be assessed with respect to biological and chemical risks. Immunogenic substances have been listed as the products of nanocelluloses, regulated by the source and surface functionality [68], which must be thoroughly assessed to ensure safe biomedical translation.

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6. Conclusions

Advances in nanoengineering cellulose, the most abundant biopolymer in the world, have leveraged the controlled delivery of a broad range of bioactive cargos, including chemotherapeutic agents, anti-inflammatory drugs, and antimicrobial compounds. Nanocelluloses, commonly synthesized from the physical and/or chemical treatment of cellulose fibrils, one of the main building blocks of plant cell walls, or produced by microorganisms, such as certain bacteria, may provide cost-effective, accessible, easy to use, biocompatible, and green delivery platforms, which often benefit from hierarchical structures, ready-to-functionalize surfaces, tunable morphologies, shapes, and aspect ratios, high surface area, adjustable surface charges, mechanically strong backbone, and low or even no toxicity. Depending on the target cargo and organ, nanocelluloses may be used as individual crystals, such as CNC, bacterial CNC, and HCNC, or nanofibrils, e.g., CNF. Hydrated or dry networks of nanocelluloses with engineered mechanical properties, stability, and cargo loading/release profiles may be fabricated in the forms of hydrogels, films, and aerogels individually or combined with other polymers and nanoparticles. Besides accommodating poorly soluble drugs, nanocelluloses have been able to regulate the hydrophilicity, swelling ratio, and pH- and ion-responsiveness of carriers, which are all favorable for the effective loading and controlled release of bioactive molecules. Challenges ahead of this field encompass understanding the behavior of nanocelluloses inside the body in terms of circulation efficiency, immunomodulation, biodegradation, biodistribution, and long-term toxicity. We speculate that the emergence of the novel types of nanocelluloses, including hairy cellulose nanocrystals bearing crystalline (resistant against biodegradation) and amorphous (readily biodegradable) cellulosic regions opens new horizons for customizable cargo delivery with applications in precision medicine.

Acknowledgments

List of abbreviations

CNC	Cellulose nanocrystals
CNF	cellulose nanofibrils
BC	bacterial cellulose
HCNC	hairy cellulose nanocrystals
BNC	bacterial nanocellulose
BCNC	bacterial cellulose nanocrystals
Dox	doxorubicin
BDP	beclomethasone dipropionate

ITR	itraconazole
PBS	phosphate buffered saline
CTAB	cetyltrimethylammonium bromide
PLA	poly(lactic acid)
RBITC	rhodamine B isothiocyanate
FITC	fluorescein-5-isothiocyanate
BSA	bovine serum albumin
HSA	human serum albumin
MTX	methotrexate
5-FU	5-Fluorouracil
TH	tetracycline hydrochloride
Cur	curcumin
KT	ketorolac tromethamine
DCF	diclofenac sodium salt
ANG	angiogenin
siRNA	small (or short) interfering RNA
RPG	repaglinide
HEK-293	human embryonic kidney 293
Sf9	spodoptera frugiperda
RNase	ribonuclease
HBMEC	human brain microvascular endothelial cells
HUVEC	human umbilical vein endothelial cells
FA	folic acid
MePyrrHSO$_4$	N-methylpyrrolidinium hydrogen sulfate
Cltx	chlorotoxin
GBM	glioblastoma multiforme
MMPs	matrix metalloproteinases
DAPI	4′,6-diamidino-2-phenylindole
MCF-7	Michigan Cancer Foundation-7

AMFC	Tris(2-aminoethyl)amine
MNP	magnetic nanoparticles
XG	xanthan gum
CS	chitosan
Coll	collagen
GM	gelatin microspheres
PMC	polyelectrolyte macroion complexes
PLGA	poly (D L-lactic-co-glycolic acid)
PEI	polyethyleneimine
CMCS	carboxymethyl chitosan
pDNA	plasmid DNA
PEG	polyethylene glycol
PVA	poly(vinyl alcohol)
TGA	thermogravimetric analysis
PI	povidone-iodine
PHMB	polihexanide
LC	lethal concentration
IC	inhibitory concentration
HMP	high-methoxy pectin
Levo	levofloxacin
FuA	fusidic acid
SGF	simulated gastric fluid
SIF	simulated intestinal fluid
SEM	scanning electron microscopy
SD	sulfadiazine
IBU	ibuprofen
GO	graphene oxide
CMC	carboxymethyl cellulose
LbL	layer-by-layer

AB	antibody assay
TNF-α	tumor necrosis factor alpha
IL1-β	interleukin 1 beta
ECM	extracellular matrix
Met	metformin
Met-CNF	metformin-modified cellulose nanofiber
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
TOCNF	TEMPO-oxidized cellulose nanofibrils
PFP	platelet-free plasma
GP	glycerophosphate
P	pectin
WVP	water vapor permeability
XyG	xyloglucan-amyloid
AP	pectin from apple
HPC	hydroxypropyl cellulose
IC	inclusion complex
CD	cyclodextrin
SFS	sulfisoxazole

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Synopsis

Engineering certain types of microorganisms as well as the hierarchical structure of cellulose fibers, one of the main building blocks of plant cell walls, has yielded unique families of cellulose-based nanomaterials, which have leveraged the effective delivery of bioactive molecules.
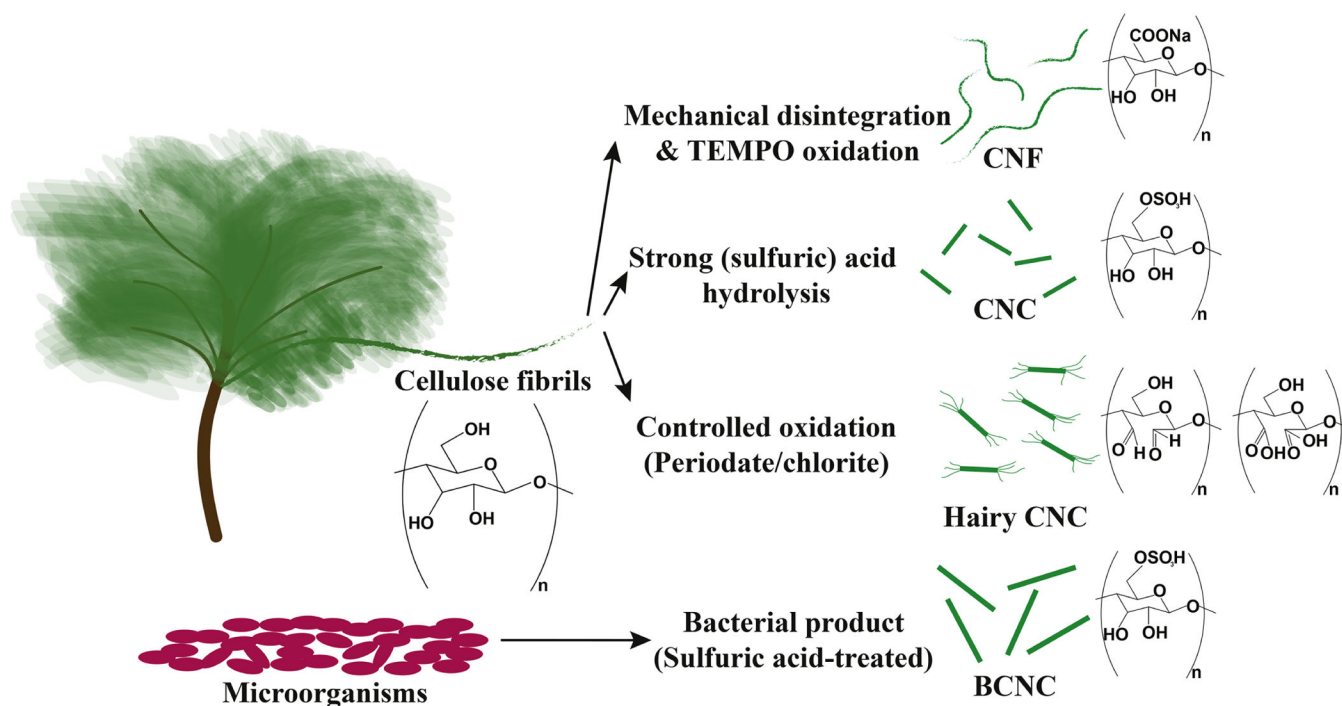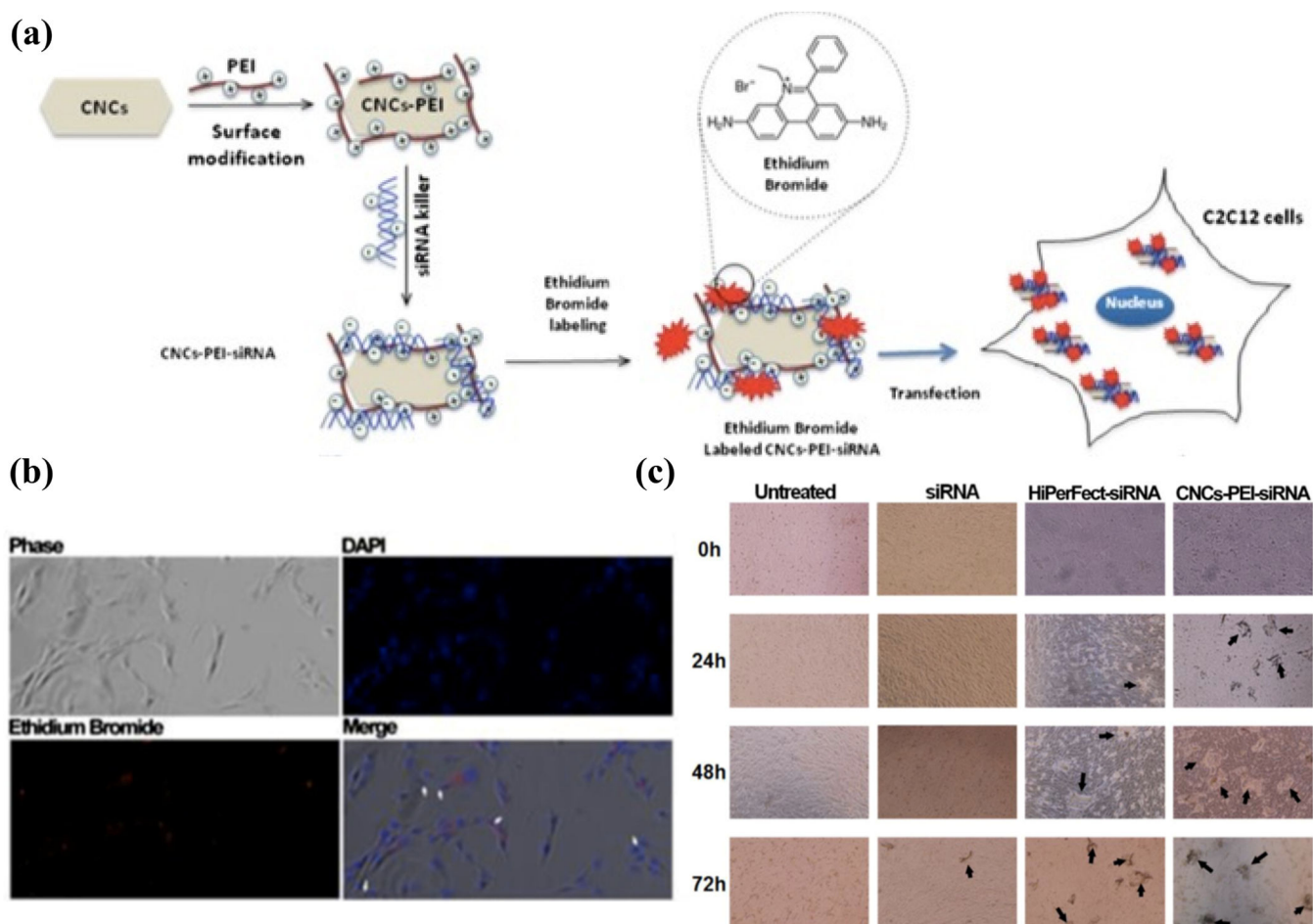
Figure 1.
Nanocelluloses and examples of their production methods. Plants and microorganisms are two main sources of cellulose. Cellulose fibrils (often chemically pre-treated, e.g., with TEMPO) are disintegrated into nanoscale fibrils, named cellulose nanofibrils (CNF) through rigorous mechanical processing (such as microfluidization and high-pressure homogenization) [13]. When cellulose fibrils are treated with a strong acid, such as sulfuric acid, the amorphous regions of fibrils are hydrolyzed, yielding needle-shaped crystals known as cellulose nanocrystals (CNC) [17]. Moreover, instead of removing amorphous regions, when they are solubilized through oxidation, cellulose fibrils fall apart into CNCs sandwiched between two protruding layers of functionalized cellulose chains. This class of newly emerged nanocelluloses is called hairy CNCs [8,9,18]. Some microorganisms, such as bacteria *Acetobacter xylinum*, also produce cellulose with a high purity, which may further be processed with a strong acid to yield bacterial CNCs (BCNC) [14,16]. Note that other sources of nanocelluloses are tunicates and algae [19].
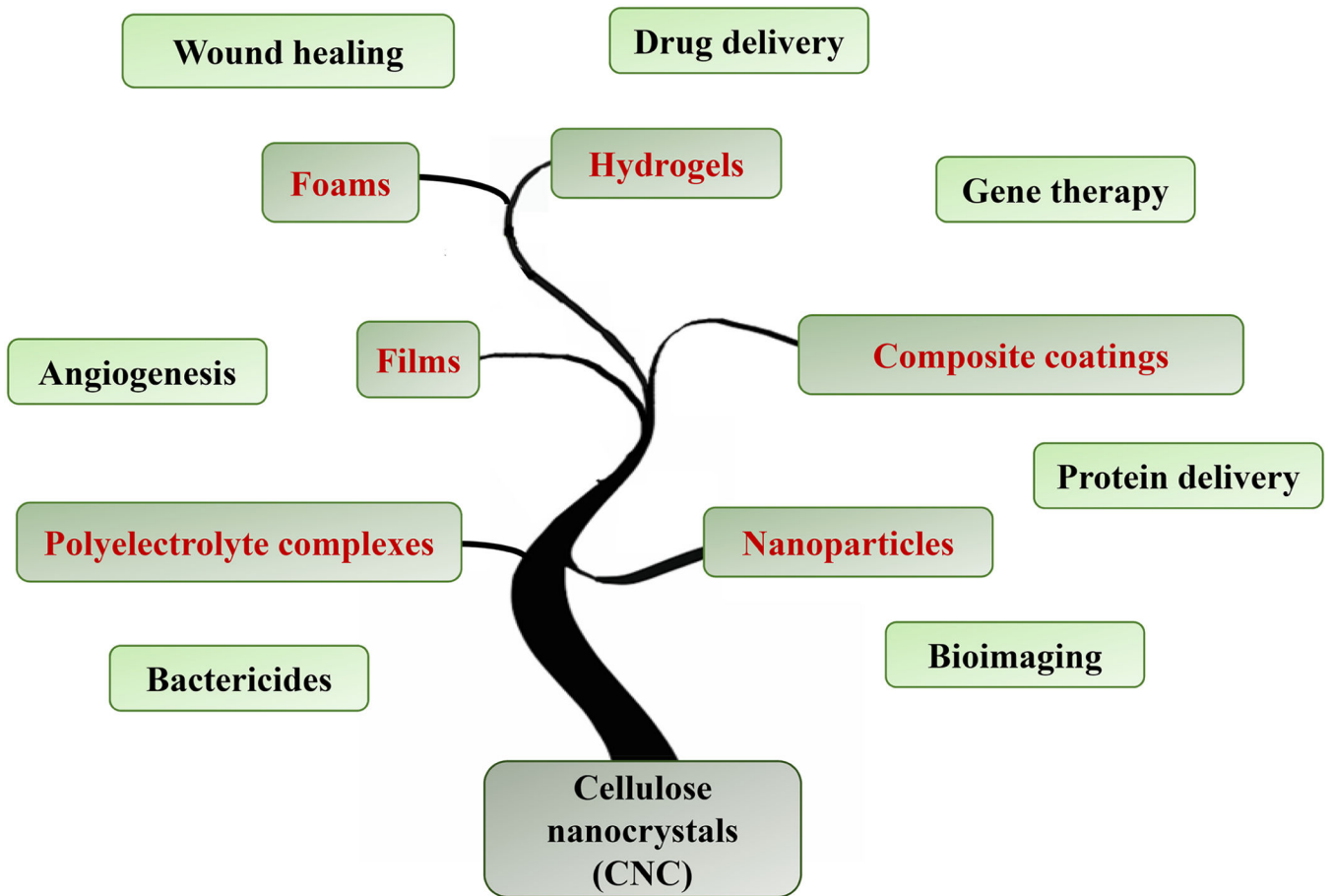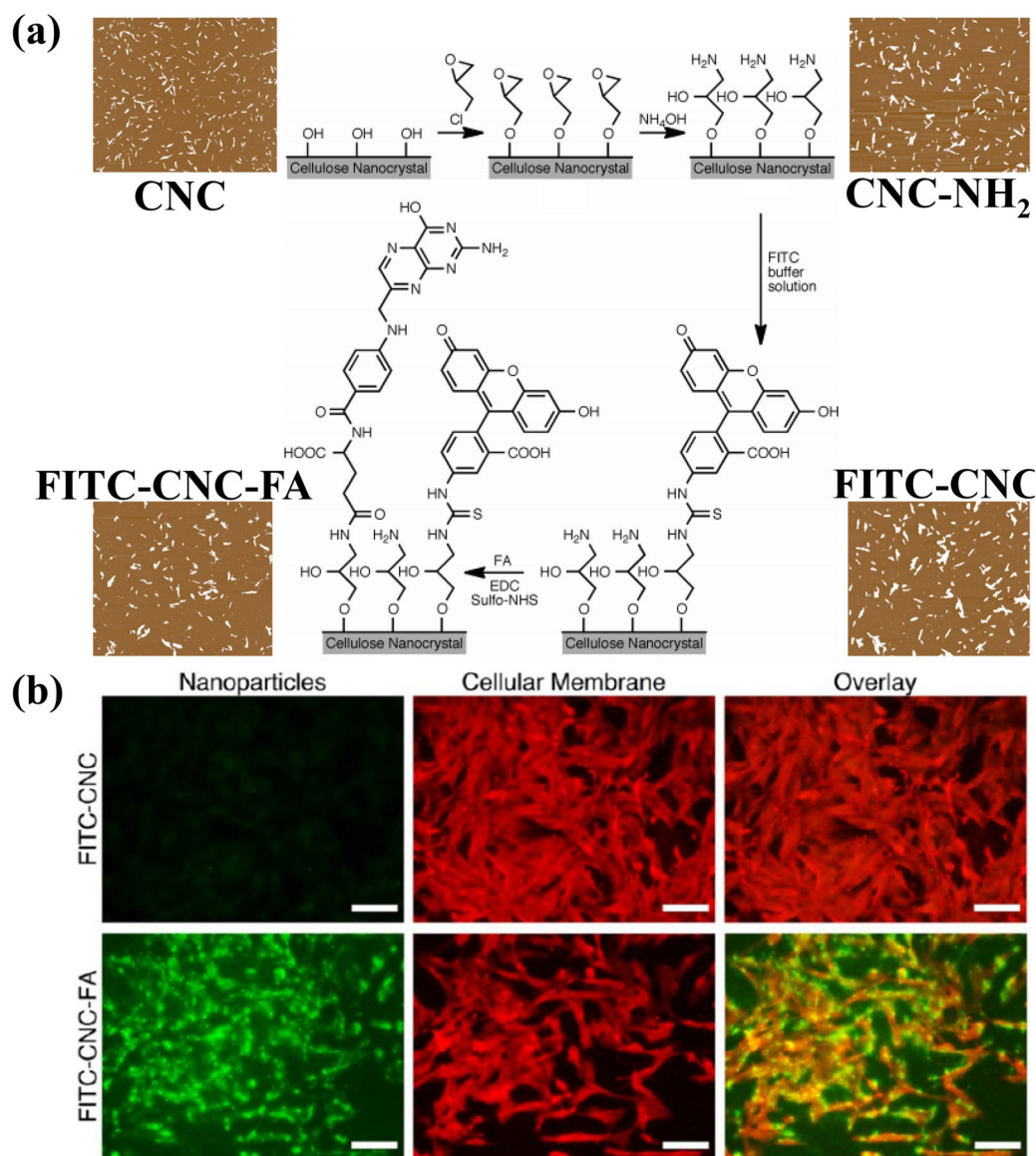
Figure 2.
Various types of cellulose nanocrystal (CNC)-based biomaterials for nanomedicine and their main applications.
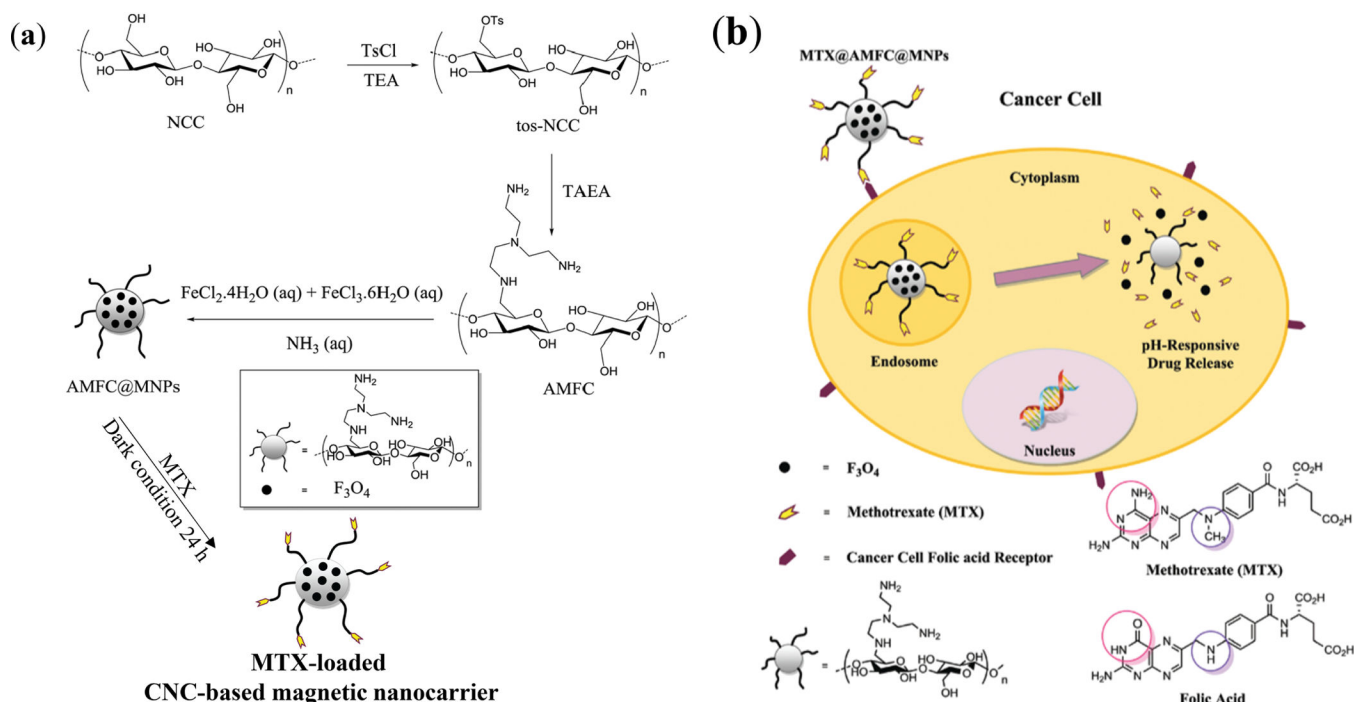
Figure 3.
(**a**) CNCs are readily decorated with polycations (e.g., polyethylenimine, PEI), imparting a positive charge to their surface, which promotes the electrostatic (physical) binding of siRNA killer. These self-assembled nanoparticles are internalized by C2C12 myoblasts (**b**), resulting in gene editing, silencing the expression of cell cycle genes, and promoting apoptosisdriven cell death [87]. (**c**) Within 24–72 h, cell death becomes evident by the formation of debris (shown with arrows), which is more pronounced for CNC-PEI-siRNA complexes, similar to the commercial transfection reagent (HiPerFect, Qiagen). Carbohydr. Polym., 164: 258–267, Copyright (2017), with permission from Elsevier.
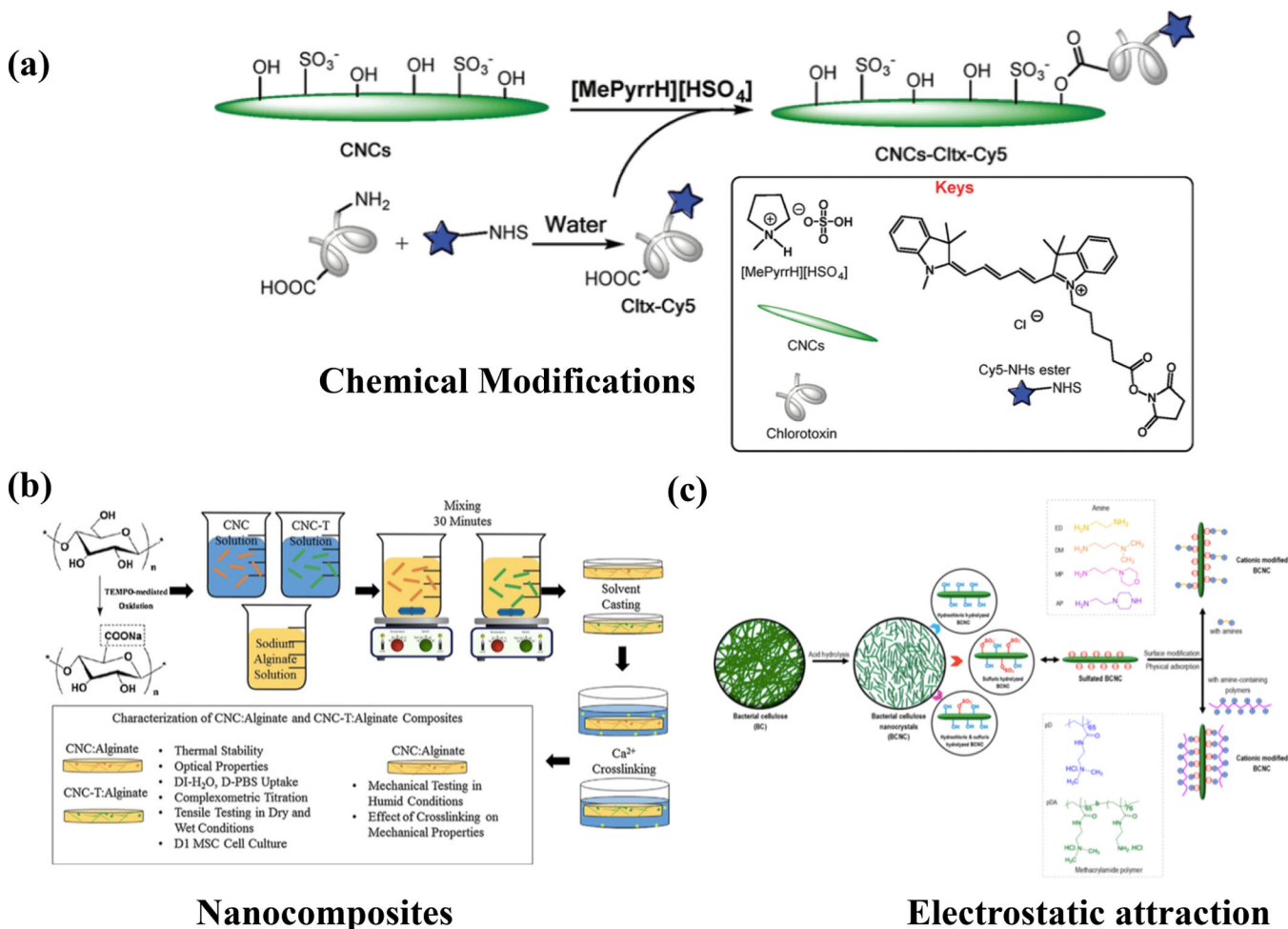
Figure 4.
(**a**) Chemical modification of CNC surface with FITC and folic acid (FA) for visualizing the enhanced receptor-mediated uptake of the nanocelluloses by human DBTRG05MG, which is shown in (**b**) the fluorescence images of stained cells [80]. Reprinted with permission from S. Dong, H.J. Cho, Y.W. Lee, M. Roman, Synthesis and cellular uptake of folic acid-conjugated CNCs for cancer targeting. Reprinted with permission from (Biomacromolecules, 15:1560–1567). Copyright (2014) American Chemical Society.
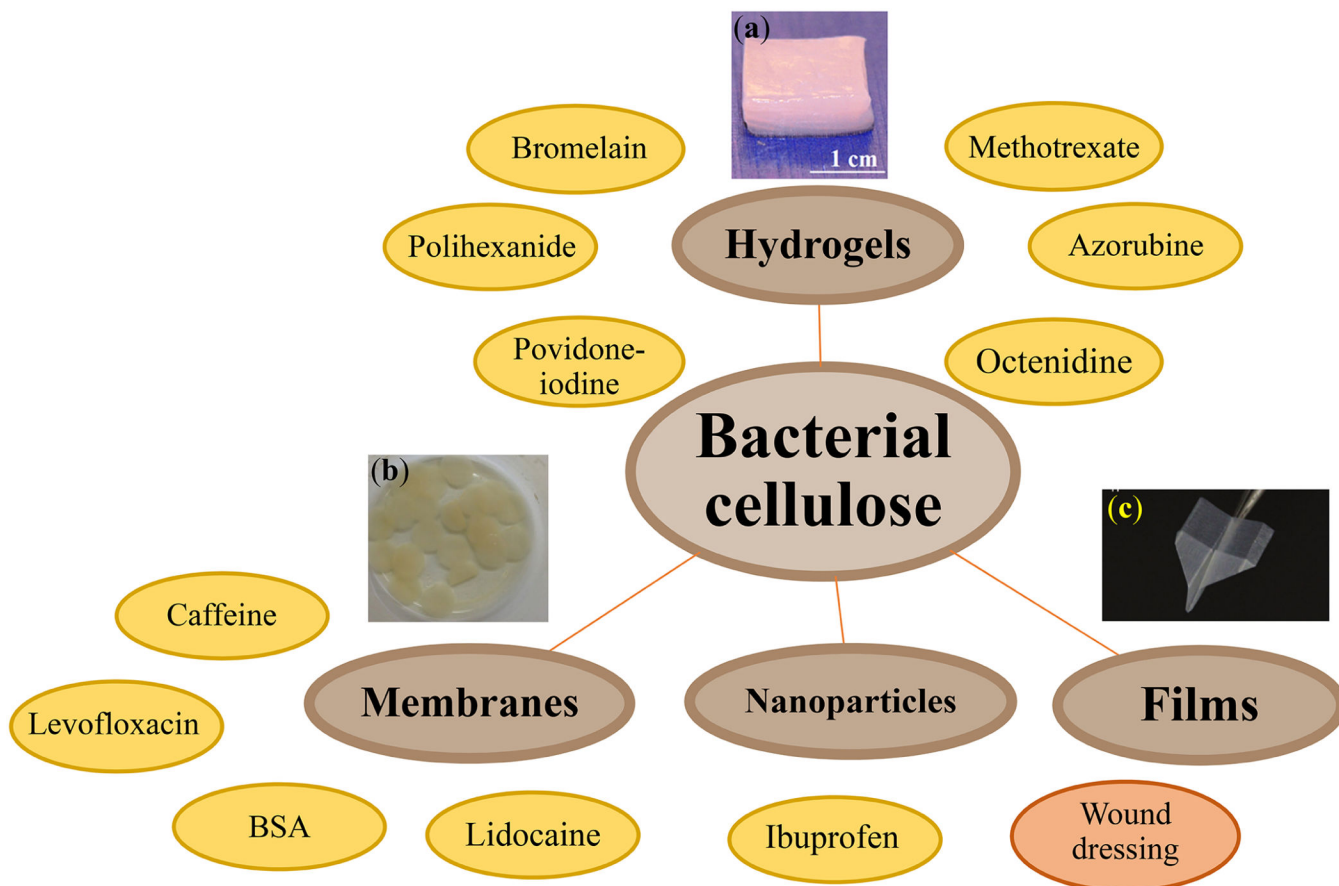
Figure 5.
(**a**) Synthesis of pH-responsive magnetic CNC-based nanocarrier. The nanocellulose was reacted with tosylchloride for the functionalization with tris(2-aminoethyl)amine (AMFC), providing amino groups to effectively attract the carboxyl groups of methotrexate (MTX, an anticancer drug). The magnetic CNC-based MTX delivery system, fabricated by an *in situ* reaction with FeCl$_2$ and FeCl$_3$, provides AMFC-coated magnetic nanoparticles (AMFC@MNPs), which are responsive to pH. (**b**) MTX-loaded magnetic CNC carriers, benefitting from the similarity of MTX to folic acid, faciliatet the folate receptor-mediated cell internalization [82]. Reproduced from [82] with permission from the Centre National de la Recherche Scientifique (CNRS) and The Royal Society of Chemistry.

(a)

Chemical Modifications

(b)

Nanocomposites

(c)

Electrostatic attraction

Figure 6.
Examples of common methods to make cellulose nanocrystal (CNC)-based cargo delivery systems. (**a**) Direct chemical modifications of CNC surface to conjugate Cltx via the Fisher esterification of the hydroxyl groups of CNC and the carboxylic acid groups of a drug in a Brønsted acid ionic liquid [90], reproduced from [90] with permission from the Centre National de la Recherche Scientifique (CNRS) and The Royal Society of Chemistry. (**b**) Solvent casting of CNC-alginate nanocomposites crosslinked using calcium ions. Carbohydr. Polym., 1:186–195, Copyright (2018), with permission from Elsevier [126]**.** This platform may be used to encapsulate active molecule of choice. (**c**) Self-assembly through the electrostatic adsorption of sulfuric acid-hydrolyzed CNC (bacterial) with amine-bearing polymers [127]. Reprinted with permission from [127]. Copyright (2018) American Chemical Society.
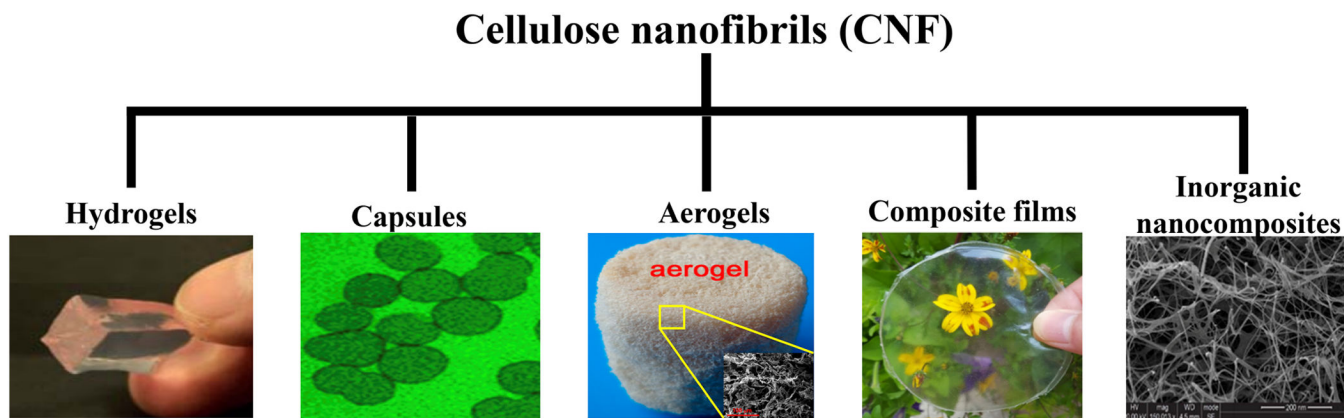
Figure 7.
Bacterial cellulose (BC)-based biomaterials, including colloidal nanoparticles, films, membranes, and hydrogels for the delivery of a broad range of cargos. Panel (**a**) is reprinted with permission from [146], Copyright © 2015, Springer Nature, Springer Science Business Media New York. Panel (**b**) is reprinted from [140] under Creative Commons Attribution 4.0 International License for the Open Access content. Panel (**c**) is reprinted with permission from [147]. © 2018 WILEY- VCH Verlag GmbH & Co. KGaA, Weinheim.
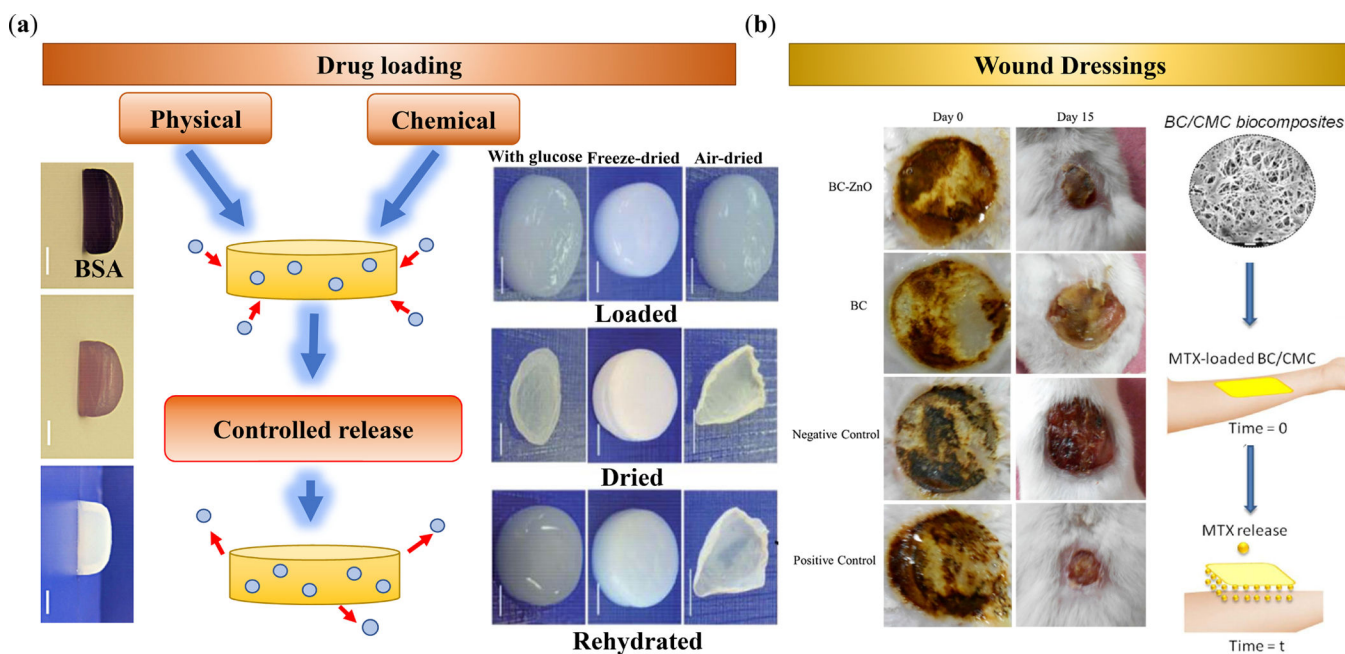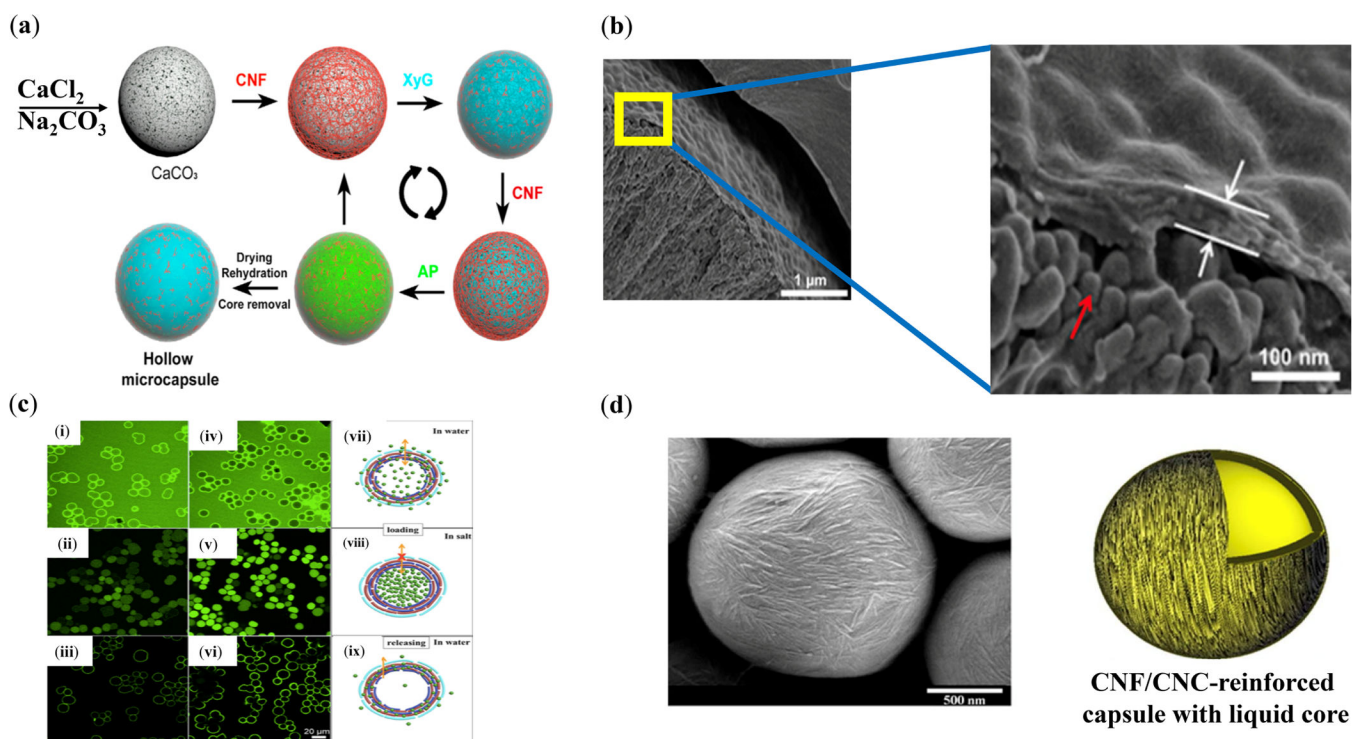
Figure 8.

(a) Mechanisms of cargo (e.g., BSA [136]) loading in BC-based carriers, including physical absorption/adsorption and chemical conjugation. The carrier may be used in hydrated or dry states. Supplementing BC with additives, such as glucose, provides remarkable shape recovery after rehydration [135]. **(b)** BC-based materials have been widely used for wound dressing, enabling the controlled release of bioactive (e.g., MTX) molecules [143]. BC may also be combined with antibacterial nanomaterials, such as ZnO for burn wounds [128]. Images in Panel (**a**, left) are reprinted from [136] Copyright (2014), with permission from Elsevier. Images in panel (**a**, right) are reprinted from [135] under Creative Commons Attribution 3.0 Unported Licence. Panel (**b**, left) is adapted from [128] Copyright (2017), with permission from Elsevier. Panel (**b**, right) is adapted from [143] Copyright (2018), with permission from Elsevier.

Cellulose nanofibrils (CNF)

Hydrogels Capsules Aerogels Composite films Inorganic nanocomposites

Figure 9.
Cellulose nanofibril (CNF)-based biomaterials, including hydrogels, aerogels, capsules, films, and inorganic nanocomposites for cargo delivery. Images of hydrogel, capsules, aerogel, composite films, and inorganic nanocomposites are reprinted from [193], [188], [194], [195], and [185] with permissions from The Royal Society of Chemistry, Copyright (2017) American Chemical Society, Copyright (2015) American Chemical Society, Copyright (2017) American Chemical Society, and Elsevier, respectively.
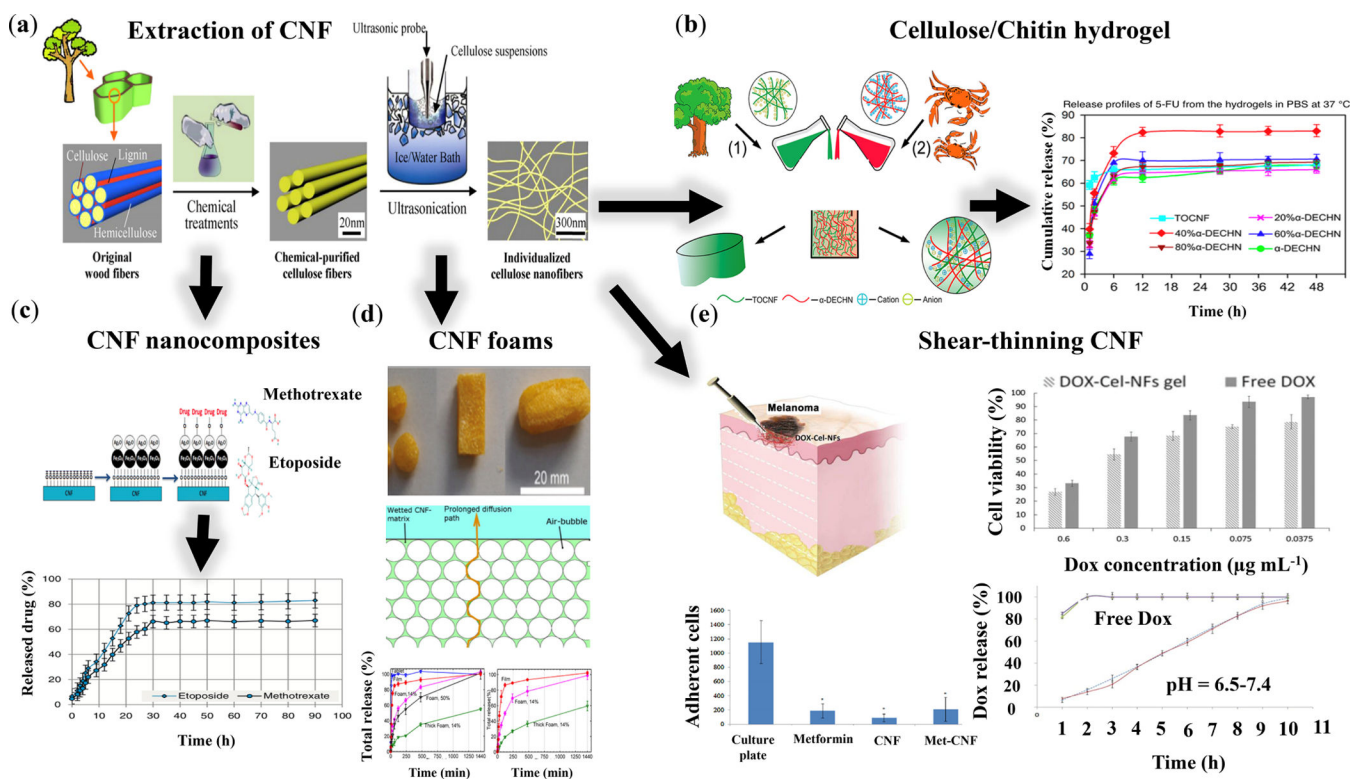
Figure 10.
Cellulose nanofibril capsules. (**a**) Fabrication of CNF microcapsules from colloidal CNF dispersions using sacrificial calcium carbonate cores undergoing the layer-by-layer (LbL) assembly of CNF, XyG, CNF, and AP [187]. (**b**) The cross section of capsules [188], indicating a calcium carbonate core (red arrow) coated with the multilayer CNF nanocomposite (white arrows). (**c**) Capsule pores change size as a response to ionic strength variation, providing a stimuli-responsive cargo carrier platform with tailored loading (in water)/trapping (in salt)/release (in water) capacities [187]. (**d**) Mechanically-stable liquid-core capsules [205] made up of a covalently crosslinked CNF/CNC shell with an inner aromatic polyurea layer. These capsules are about six times stiffer than aromatic polyurea capsules. Images of panels (**a**) and (**c**) are reprinted from [187], copyright (2018), with permission from Elsevier. Panel (**b**) is reprinted with permission from [188]. Copyright (2017) American Chemical Society. Panel (**d**) is reprinted with permission from [205]. Copyright (2014) American Chemical Society.
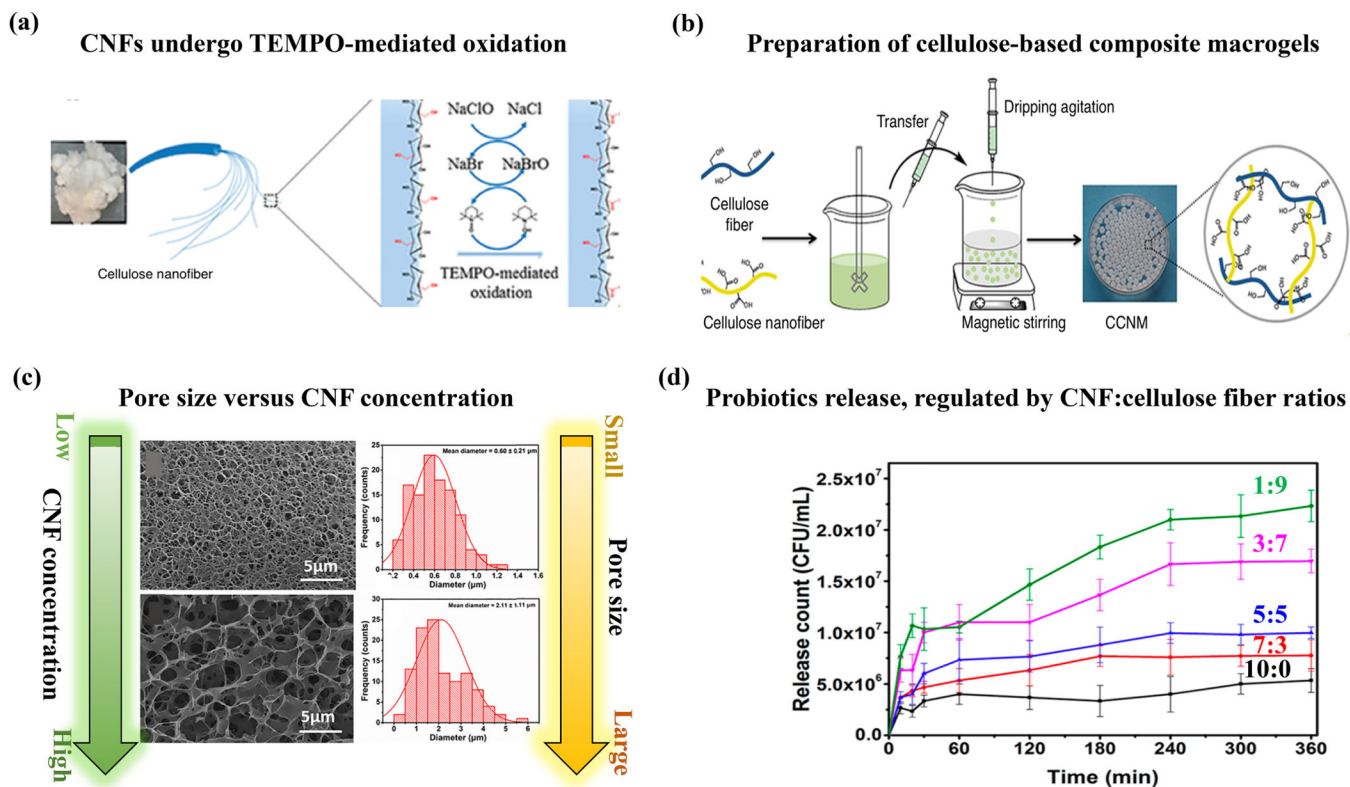
Figure 11.

CNF-based hydrogel beads for the controlled release of probiotics. (**a**) Cellulose fibrils undergo TEMPO-mediated oxidation, resulting in the carboxylate conjugation to the C6 of monomers (glucose). (**b**) The oxidized nanofibrils are mixed with cellulose fibers in an aqueous solution containing LiOH/urea/water and maintained at −20°C, followed by freeze-thawing and centrifugation. The product is added to a dilute hydrochloric acid solution to form the beads with various CNF-to-cellulose fiber ratios. (**c**) The bead pore size may be regulated by changing the concentration of CNF; the higher the CNF, the larger the pores. (**d**) The engineered porosity of gel beads and their pH-responsiveness originated from the carboxylic acid groups enable the controlled release of probiotics [190]. Reprinted with permission from [190]. Copyright (2018) American Chemical Society.

Figure 12.
An example of CNF production methods and CNF-based biomaterials for cargo delivery. (**a**) Extraction of CNF from trees via the physical disintegration of chemically-modified cellulose fibrils [248], Copyright (2011), with permission from Elsevier. (**b**) Hydrogels made up of carboxylated CNF and chitosan for the controlled release of 5-FU [189], reprinted with permission from [189], copyright (2018) American Chemical Society. (**c**) CNF nanocomposites for chemotherapeutic delivery via direct drug binding to Fe_3O_4-Ag_2O quantum dot-decorated CNF [185], copyright (2017), with permission from Elsevier. (**d**) CNF foams, providing a sustained release of active molecules (riboflavin) as a result of increased diffusion path [247], copyright (2016), with permission from Elsevier. (**e**) Concentrated CNF dispersions readily form a shear-thinning hydrogel, which may be used for the delivery of anti-cancer drugs, such as metformin (Met) [183] (Copyright (2017), with permission from Elsevier) and Dox [191] (Copyright (2018), with permission from John Wiley and Sons) for treating melanoma.

Table 1.

CNC-based cargo delivery: examples of recent cargos, carriers, applications, and loading and release mechanisms.

Cargo	Carrier	Potential application	Loading mechanism	Release mechanism	Year [Reference]
Rhodamine B isothiocyanate (RBITC), Fluorescein-5isothiocyanate (FITC)	Colloidal CNC	Bioimaging	Covalent conjugation	NA	2014 [80]
BSA-FITC	PLGA-doped PVA-CNC films	Biomacromolecule delivery to bone marrow	Physical entrapment	PLGA degradation	2014 [81]
MTX	Fe$_3$O$_4$ nanoparticles decorated with amino-functionalized CNC	Cancer therapy	Hydrogen bonding and electrostatic interactions	pH dependent (maximum release at pH ~ 5.4)	2016 [82]
5-FU	Xanthan gum-chitosan-CNC hydrogels	Cancer therapy	Equilibrium swelling	Non-Fickian diffusion	2017 [83]
Tetracycline hydrochloride (TH)	CNC-doped PLA/PEG nanofibers	Antibiotic	Hydrogen bonding and improved hydrophilicity	Composition regulated	2017 [84]
Curcumin (Cur)	CNC-collagen-gelatin microspheres/porous scaffolds	Wound healing	Physical trapping	Gradual dissolution	2017 [85]
Angiogenin (ANG) and Cur	PLGA/CNC/Cur/ pDNA-ANG composite nanofiber	Angiogenesis and infection prevention during wound healing	Electrostatic interactions	Expected to be pH dependent	2017 [86]
siRNA	Cationic BCNC, PEI-CNC hybrids	Gene therapy	Electrostatic interactions	pH dependent (maximum release at pH ~ 4.0)	2017 [87]
Bovine serum albumin (BSA), Human serum albumin (HSA)	CNC nanocomposites	Protein delivery for intracellular cholesterol efflux	Adsorption/chemical conjugation	pH dependent (maximum release at pH ~ 6.5–6.6)	2017 [88]
Repaglinide	Chitosan-CNC	Oral drug	Electrostatic	pH dependent	2018 [89]
(RPG)	polyelectrolyte macroion complexes (PMC)	delivery	interactions	(maximum release at acidic conditions)	
Chlorotoxin	Colloidal CNC	Brain tumor therapy	Covalent conjugation	NA (internalization)	2018 [90]

Table 2.

Cargo delivery systems based on BC: carriers, applications, and loading and release mechanisms

Drug/bioactive agent	Carrier	Potential application	Loading mechanism	Release mechanism	Year [Reference]
Azorubine	Supplemented BC fleeces	Safe-to-store oral delivery and targeted drug delivery	Immersion in solution (absorption)	Diffusion	2014 [135]
Bovine serum albumin	BNC fleece	Protein delivery	High-speed vortexing	Diffusion	2014 [136]
Octenidine	BNC fleece	Wound treatment	Immersion in solution under shaking	Diffusion	2014 [137]
Octenidine	BNC/poloxamer micellar hydrogels	Dermal drug delivery	Immersion under shaking	Sustained gel dissolution	2016 [138]
Ibuprofen	BNC/graphene oxide composite hydrogels	Delivery of poorly watersoluble drugs	Immersion in drug solution	Non-Fickian, pH- regulated diffusion	2017 [139]
Bromelain	BNC membranes	Antimicrobial wound dressing	Immersion in solution under shaking	Diffusion	2017 [140]
Diclofenac sodium salt (DCF)	BNC-Poly(Nmethacryloyl glycine) membrane	Dermal and oral drug delivery	Immersion in solution	pHdependent (high release at pH ~ 7.4)	2017 [141]
NA	BNC-ZnO nanocomposite films	Antimicrobial burn wound dressing	Immersion under shaking and lyophilization	Dermal application to burn as a bandage	2017 [142]
Methotrexate	BC/CMC composite membrane	Topical treatment for psoriasis	Immersion in solution	Dissolution *in vitro*	2018 [143]
Silymarin	Zein nanoparticle/BC nanofiber composite films	Antioxidant/a ntibacterial	Encapsulatio n in nanoparticles	Improved solubility	2018 [144]
Cells (Keratinocytes and fibroblasts)	BC/acrylic acid hydrogels	burn wounds	encapsulation	NA	2018 [145]

Table 3.

Examples of recent CNF-based cargo delivery: carriers, applications, and loading and release mechanisms

Drug/bioimaging agent	Carrier	Potential application	Loading mechanism	Release mechanism	Year [Reference]
Methotrexate	Resistant starch/pectin/CNF electrospun nanofibers	Colon inflammation	Absorption and electrostatic attraction	pH dependent, enzymatic degradation	2016 [180]
Beclomethasone dipropionate, indomethacin, itraconazol	CNF microparticles and capsules	Drug delivery of poorlysoluble drugs (broad range of applications)	Mixing/filtration/drying	Diffusion	2017 [181]
Cisplatin	CNF-PVA hydrogel	Cancer treatment	Mixing	pH dependent release (maximum release at 7.4)	2017 [182]
Metformin	CNF gel	Anti-cancer (melanoma) drug delivery and metastasis prevention	Electrostatic adsorption	pH dependent	2017 [183]
N/A	Ca^{2+}-mediated crosslinked CNF hydrogel, kaolinCNF, and collagen-CNF	Hemostatic wound dressing	N/A	N/A	2017 [184]
Etoposide, methotrexate	Fe_3O_4-Ag_2O quantum dots decorated cellulose nanofibers	Chemotherapy for lung, breast, and skin cancer	Binding to Ag_2O	Drug solubility in water	2017 [185]
Bendamustine hydrochloride	CNF aerogel	Gastroretentive oral drug delivery	Absorption, electrostatic attraction	pH dependent	2017 [186]
Dextran	Microcapsules of CNF, hemicellulose, and pectin	Model drug delivery	Physical encapsulation	Ion-mediated release	2017, 2018 [187,188]
5-fluorouracil	α-chitin/TEMPO-oxidized CNF hydrogel	Anti-cancer oral drug delivery	Electrostatic adsorption through swelling	pH and swelling dependent	2018 [189]
L. plantarum	Cellulose fiber and nanofibril hydrogel macroparticle	Probiotic delivery	Physical encapsulatio n	pH responsive pore size	2018 [190]
Doxorubicin	Injectable CNF gel	Cancer (Melanoma) treatment and metastasis prevention	Electrostatic attraction	pH dependent	2018 [191]
Tetracycline hydrochloride	CNF/ polydopamine hydrogel	Wound healing	Electrostatic attraction	pH dependent, high release at low pH	2018 [192]