CHAPTER

9

Nanocellulose: a sustainable nanomaterial for controlled drug delivery applications

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9.1 Introduction

9.1.1 Evolution of controlled drug delivery

Conventional drug delivery approaches have typically involved direct injection to the appropriate site, whether it be subcutaneous, intradermal, intramuscular, epidermal, intrathecal, intrasynovial, intravenous, and intra-arterial. Alternatively, therapeutic drugs have also been administered through mucosal membranes, including buccal, sublingual, gingival, rectal, vaginal, nasal, and pulmonary routes. Otherwise, conventional drug administration involves oral ingestion with liquids or tablet formulations, transdermal patches, and ocular contact lens. However, many or all of these administration routes face the following problems that impact the acceptance and effectiveness of the delivered drugs:

1. Accurate drug dosage to the target site;
2. Limiting off-target side effects;
3. Controlling drug release kinetics;
4. Protection against physiological barriers that limit drug infiltration;
5. Masking the taste of offensive drugs;
6. Providing amenable preparations for hard to administer to patients;

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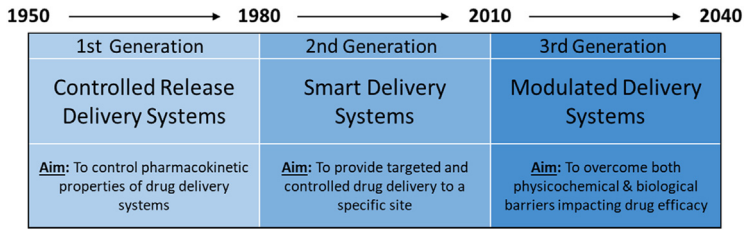

FIGURE 9.1 Evolution of controlled drug delivery systems (Yun, Lee, & Park, 2015). Source: *Adapted from Yun Y. H., Lee B.K., Park K. (2015) Controlled drug delivery: Historical perspective for the next generation.* Journal of Controlled Release: Official Journal of the Controlled Release Society 219:2–7. *https://doi.org/10.1016/j.jconrel.2015.10.005.*

7. Protection against environmental degradation during storage.

Since the middle of the 20th century, controlled drug delivery systems have been developed with the aim of mitigating these challenges, especially accurate drug delivery to a specific target site in a controlled manner with limited side effects to the rest of the body. From 1950–1980, the first generation of drug delivery systems focused on controlling the physicochemical properties of drug release, which typically involved oral or transdermal formulations. Sustained drug release systems were successfully developed for many clinical applications, despite a facile understanding of the biological barriers impacting drug distribution and efficacy (Fig. 9.1).

The development of second-generation smart drug delivery systems aimed at more sophisticated control over drug delivery, including long-term sustained release, autotargeted delivery, and self-regulated release. However, their efficiency in the in vivo setting was limited by biological barriers within the body, including the inactivation by local cells and proteins, nonspecific accumulation in healthy organs, blood flow and fluid dynamic limitations, unfavorable pressure gradients, cellular internalization and the action of drug efflux pumps (Blanco, Shen, & Ferrari, 2015). Ultimately, these biological barriers limited bioavailability at the desired site, impacting the in vitro to in vivo correlation (IVIVC) of these drug delivery systems—drugs discovered and developed through in vitro trials failed to present sufficient efficacy and safety at the pre-clinical development stage.

> *"IVIVC has not been found for most parenteral formulations of biotech drugs, making it difficult to predict the* in vivo *bioavailability from the* in vitro *release profiles"* (Yun et al., 2015).

The 3rd generation of controlled drug delivery systems must continue to overcome physicochemical barriers to effective drug delivery, such as the delivery of poorly water-soluble molecules, peptides, proteins, and nucleic acids, while considering these challenges in the context of the existing biological barriers. Integrated solutions may be achieved through the development of advanced functional materials, nanotechnology-enabled delivery technologies that control drug bioavailability (crossing endothelial barriers—e.g., blood-brain barrier), biodistribution (accumulation and retention throughout the body), and appropriate drug release timescales to promote therapeutic safety and efficacy.

Considering these challenges, the pipeline of medical and pharmaceutical products making it to market remains low and slow, especially for formulations that include highly

lipophilic or high molar mass drugs. While advances in the understanding of disease pathophysiology have shown promise for developing effective treatment systems, control over the temporal, and spatial localization of drug molecules remains a fundamental challenge limiting efficacy. In addition, difficulty scaling up drug development to a commercially viable product, associated with limited IVIVC and high cost, is a considerable challenge for the drug development pipeline (Klemm, Cranston, & Fischer, 2018).

9.1.2 Significance of controlled drug delivery

The improving resolution of disease modeling is leading to a gain in knowledge surrounding the spatial and temporal organization of pathophysiological states within the human body. However, this knowledge goes underutilized without sophisticated methods to treat these uncovered disease conditions, such as with the use of targeted drug delivery to specific cell types or regions in the body, as outlined in the previous section. Advanced drug delivery systems can unlock the therapeutic benefits of the improved disease understanding, enabling significant healthcare advances. Engineering controlled drug delivery systems is required to transform discovered drug candidates with significant drawbacks, such as poor water solubility and low biostability, into effective drug formulations for diseases impacting healthcare now and into the future.

In addition, the ability to target drug delivery to specific cell types or bodily regions reduces systemic drug exposure, therefore reducing the degree of unintended side effects from off-target drug interactions. As a result, a higher drug concentration can be delivered to the target site relative to the rest of the body for the same dosage concentration, improving safety and efficacy of the treatment. Alternatively, a lower concentration dose can be administered to the patient for a similar therapeutic effect, reducing production cost for the treatment and economic burden for the patient.

Furthermore, controlled drug delivery has allowed for improved functionality for therapeutic agents. In terms of spatial functionality, drugs can be targeted toward difficult to reach locations within the body, such as bypassing the blood-brain barrier, which were previously inaccessible without invasive surgical techniques (Scherrmann, 2002). Considering temporal functionality, controlled drug delivery systems are being developed to respond to physiological cues of the cellular microenvironment, such as stimuli-responsive capabilities. This provides the ability to design drug release profiles that are tailored to the context of the tissue or disease in focus.

Extending beyond the scope of drug delivery, controlled drug delivery systems have the potential for being functionalized into biosensor and theragnostic devices, aiding in the development of early diagnostic and preventative medicine technologies for improved long-term healthcare outcomes.

9.1.3 Hydrogels for controlled drug delivery

A class of biomaterial extensively used for controlled drug delivery is that of hydrogels. Hydrogels are polymeric materials with a high water sorption capacity (i.e., hydrophilic) and the ability to maintain a stable three-dimensional (3D) structure once swollen (Villalba-Rodríguez, Dhama, & Iqbal, 2017). Due to their highly porous 3D structure,

hydrogels are capable of storing therapeutic molecules within their porous polymeric matrix, and subsequently controlling their rate of release through a range of mechanisms once implanted within or on the body (Shojaeiarani, Bajwa, & Shirzadifar, 2019). Hydrogel porosity can be adjusted through choosing different types of polymeric material, changing the concentration of the cross-linking agent, altering the hydrogel fabrication method, or changing the material's swelling capacity through the choice of solvent type, among other options. Controlled drug release can also occur in response to environmental triggers, in the case of stimuli-responsive (bioresponsive) drug delivery systems.

The following requirements and considerations are important for the development of controlled drug delivery hydrogel systems:

1. *Mechanical properties* of the hydrogel ensure structural integrity of the material and has an influence on control of the drug release rate. A hydrogel that is prone to mechanical deformation is at risk of variable control in drug release. This is closely related to control of the hydrogel *degradation rate*.
2. The *drug-loading* process must be feasible in terms of time and scalability. Similarly, control over the *drug retention and release* profile is essential for safe and efficacious drug delivery.
3. Hydrogel rheology is important for the administration of the material, especially considering injectable hydrogel systems.
4. The type of environmental trigger for stimuli-responsive drug delivery, to ensure appropriate timing and magnitude of drug release.
5. All throughout this process, the fabrication of modern hydrogels must be achieved through *facile*, *scalable*, and *cost-effective synthesis* methods, while ensuring that *quality control* and replicability of the process.
6. All components involved throughout hydrogel production, especially in the final product, must be *safe* and present negligible cytotoxicity to living tissue.

With these parameters in mind, early hydrogel development of involved synthetic materials due to the demand for high water uptake, processability, and long service life. Research prioritization is now shifting toward the development of sustainable, biobased material alternatives, while aiming to maintain the advantageous properties of synthetically derived materials.

9.1.4 Biobased hydrogel materials

Biobased materials exhibit potential across a wide range of biomedical applications as a result of their general biocompatibility, nontoxicity, and abundance. In addition, a subset of these materials has the capability to self-assemble into organized, highly hydrated 3D network structures. Biobased polymers that are applicable for drug delivery include polylactic (glycolic) acid (PLA/PLGA), alginate, chitosan, collagen, and cellulose. A detailed comparison of the properties, limitations and applications of these biobased materials can be found elsewhere (Shojaeiarani et al., 2019).

Considering these materials, cellulose presents itself as an attractive candidate due to its wide availability, renewability, biocompatibility, nontoxicity, impressive mechanical properties, high surface area, high hydrophilicity and swelling capacity, sustainability, facile preparation, and potential for surface functionalization.

9.2 Nanocellulose-based hydrogels

9.2.1 Nanocellulose

Nanocellulose can be produced in a range of different forms, depending on the source material, processing method, and hydrogel fabrication method. Cellulose is contained within the cell wall of all plants on Earth, as well as being biosynthesized from bacteria and some aquatic organisms. Wood has been the primary source of cellulosic biomass over the past century, but nonwood alternatives such as agricultural residues and industrial waste have increasingly been used in the past decade (Pennells, Godwin, Amiralian, & Martin, 2019).

Considering the fabrication of a nanocellulose hydrogel system, bacterial biosynthesis is a bottom-up approach, whereas the decomposition of the plant cell wall structure is a top-down approach. Top-down processing is the predominant method for nanocellulose production from plant, animal or algae-based biomass. This decomposition process can be achieved through a wide range of chemical, mechanical, enzymatic, or other treatments. The choice of treatment type, or a combination thereof, depends upon the requirements of the final product (Mondal, 2019).

9.2.1.1 Bacterial nanocellulose

Nanocellulose synthesized from bacteria (BNC) are produced in the form of a water stable nanofiber network (1% BNC, 99% water), with fibers ranging between 20–100 nm in diameter (Klemm et al., 2018). Due to this biosynthesis method, BNC has advantageous attributes of high surface area, a high degree of polymerization, high wet tensile strength, purity, and high crystallinity. In addition, BNC is recognized as highly biocompatible, potentially due to the flexibility and porosity of this material resembling the physical properties of collagen in the extracellular matrix (ECM) (Abeer, Mohd Amin, & Martin, 2014). Also, the stability of BNC at a wide range of temperatures allows for facile heat sterilization, an important step in biomaterial production. Progress is being made in the scalability of BNC production through enhanced control of the culturing conditions and the properties of the starting bacterial strain. However, cost is a significant limiting factor to the scalability of BNC production. A detailed overview of different types of BNC hydrogels, their challenges and the proposed mechanisms to overcome them have been described elsewhere (Picheth, Pirich, & Sierakowski, 2017; Salimi, Sotudeh-Gharebagh, & Zarghami, 2019).

9.2.1.2 Cellulose nanocrystals

Cellulose nanocrystals (CNC)—otherwise known as cellulose nanowhiskers (CNWs)—are short, stiff, rod-like particles that are typically produced from strong acid hydrolysis. Acid hydrolysis treatment of lignocellulosic biomass involves hydrolytic attack of the amorphous regions of the cellulose fibers, while retaining the crystalline regions with higher resistance to hydrolytic degradation. These crystalline portions of cellulose fibers that are retained after acid hydrolysis are CNCs, with a typical fiber diameter of 3–10 nm and an aspect ratio of 5–50 (Tappi, 2013). CNCs have excellent mechanical properties, including tensile strength and stiffness, which underlies their past utilization as a nanocomposite strength additive for a wide range of materials, including polymeric biomaterials (Kargarzadeh, Mariano, & Huang, 2017). An extensive outline of the treatment processes used for the production of CNCs is available elsewhere within the literature (Kargarzadeh, Mariano, & Gopakumar, 2018).

9.2.1.3 Cellulose nanofibers

Cellulose nanofibers (CNFs)—otherwise known as cellulose nanofibrils—are produced from top-down decomposition of lignocellulosic biomass. CNFs are typically described as long, flexible, rope-like fibers with both crystalline and amorphous regions. Standardized terminology has proposed that CNF should have a diameter of around 5–30 nm, but with an aspect ratio greater than 50 (Tappi, 2013). Processing of lignocellulosic biomass into CNFs involves disruption of the composite biochemical matrix of the plant cell wall, partial digestion of structural components such as hemicellulose and lignin, and changes to the surface functionality of cellulose fibers. Treatments are employed to overcome the intermolecular forces between cellulose fibers, while preserving fiber length through maintaining the amorphous regions of the fibers. In this context, the decomposition of native cellulose bundles into CNFs—a process otherwise referred to as fibrillation—represents a more sustainable method for nanocellulose production, due to the lower intensity of chemical and mechanical processing. An extensive outline of the treatment processes used to produce CNFs is available elsewhere within the literature (Nechyporchuk, Belgacem, & Bras, 2016; Osong, Norgren, & Engstrand, 2016).

9.2.2 Benefits of cellulose-based nanohydrogels

9.2.2.1 Abundance and renewability

An inherent advantage of biobased nanocellulose hydrogels is the abundance and renewability of the raw material. Cellulose is the most abundant organic compound on Earth and the major structural component of every plant's cell wall (Gardner & Blackwell, 1974). The ability to continuously regrow plant or bacterial biomass enables cellulose renewability, and the carbon sequestration involved in the production of biomass-derived hydrogel material contributes to sustainability.

9.2.2.2 High hydrophilicity and swelling capacity

The high degree of hydroxyl groups decorating the surface area of nanocellulose fibers, as discussed in previous sections, contributes to the water sorption capability of this material.

> *"Hydrogels are water-absorbing natural or synthetic polymeric substances, which swell in water and retain a significant amount of water within the structure without dissolving in water"* (Mondal & Haque, 2019)

While the high hydrophilicity and swelling capacity of nanocellulose has conventionally introduced challenges for the processing and performance of CNC- or CNF-reinforced plastic nanocomposites, this property is the core value proposition for their use as hydrogel materials (De France, Hoare, & Cranston, 2017). Swelling capacity constitutes a hydrogel's capability to water, accept and store therapeutic molecules, and control the rate of molecular migration and dissolution.

The degree of swelling is dictated by the balance between the solvent's osmotic force, which drives solvent migration within the voids of the polymeric matrix, and the opposing elasticity force preventing excess deformation of the polymeric matrix itself (Mondal & Haque, 2019). The point of equilibrium between these forces is therefore the value of maximum swelling

capacity. Optimum hydrogel swelling capacity for drug delivery applications aims to load the maximum drug cargo into the system and control its release at the appropriate time or in the right environment, without compromising the hydrogel's mechanical stability.

9.2.2.3 High surface area

Cellulosic biomass can undergo chemical and mechanical treatment to significantly increases their specific surface area and generate nanocellulose; this process is known as fibrillation. Nanocellulose exhibit a high specific surface area of up to several hundreds of m^2/g, contributing to the formation of a mechanically stable hydrogel. Mechanical stability is inherently higher for CNFs over CNCs due to their higher aspect ratio and higher degree of fiber entanglement. The high surface area of nanocellulose, and the corresponding porous network structure, provides a higher drug-loading capacity in their self-assembled hydrogel state (Du, Liu, & Zhang, 2019).

9.2.2.4 High surface functionality

The large surface area of nanocellulose fibers coated in reactive hydroxyl groups enables a high degree of fiber surface functionalization (Sheikhi, Hayashi, & Eichenbaum, 2019). This tailoring of the fiber surface chemistry holds wide potential for functional modification of nanocellulose and their nanocomposite materials for advanced drug delivery systems.

> *"One of the most specific characteristics of cellulose is that each of its glucose unit bears three hydroxyl groups, which endows nanocellulose a reactive surface covered with numerous active hydroxyl groups"* (Lin & Dufresne, 2014)

Surface functionalization can enhance mechanical stability of the hydrogel, improve control over drug loading, drug release profiles and target site localization, or enable bioresponsiveness to environmental cues within the body.

The resulting surface properties of functionalized hydrogels play a crucial role in the in vivo performance of the material. Surface functionalization has an impact on wettability, topography, chemistry, surface charge, the degree of hydrophobic and hydrophilic domains, and hydrogel density, which significantly influences cell-material interaction (Klemm et al., 2018). A comprehensive overview detailing the range of chemical modification possible for nanocellulose can be found within the literature (Habibi, 2014).

9.2.2.5 Mechanical stability

The mechanical properties of single BNC fibers have been estimated by different techniques, with their elastic moduli being reported as 78 GPa from atomic force microscopy and 114 GPa from Raman spectroscopy. The high moduli of BNC is related to its high crystallinity in comparison to plant-derived CNF (Abeer et al., 2014).

However, the most important property of hydrogel materials is their mechanical stability in hydrated form. The fibrillar network of BNC enables a high wet tensile strength, relating to the wet state in which it is biosynthesized. In addition, the nondegradability of BNC hydrogels under standard physiological conditions allows it to maintain long-term mechanical and chemical stability (Jorfi & Foster, 2015). Similarly, the previously mentioned high surface area of CNC and CNF hydrogel results in the formation of mechanically stable self-assembled structures (Du et al., 2019).

9.2.2.6 Sustainability and facile preparation

In addition to the availability and renewability of cellulosic raw materials, there are advantages associated with the sustainable processing of this biomass and facile preparation of nanocellulose hydrogels. Sustainability in biomass processing is becoming a major focus for the nanocellulose field, subject to innovation in treatment types, processing efficiency, and sustainable biomass procurement (Pennells et al., 2019).

In addition to benefits of the starting biomass, tuning of the nanocellulose surface properties and the preparation of nanocellulose-based hydrogels involves a relatively facile methodology (Grishkewich, Mohammed, Tang, & Tam, 2017; Hosseinmardi, Annamalai, & Martine, 2018) The extensive hydroxyl groups decorating the surface of nanocellulose provides a versatile platform for chemical functionalization to produce hydrogel materials with unique properties. Nanocellulose also offers advantageous thermal, mechanical, rheological and properties that limit processability challenges (Villalba-Rodríguez et al., 2017).

9.2.2.7 Biocompatibility

Bacterial cellulose is recognized as highly biocompatible, due its high purity and mechanical properties, which resemble that of the ECM (Abeer et al., 2014). BNC biocompatibility has been reported across a range of in vitro and in vivo studies in mice, rats, rabbits, pigs, and humans (Klemm et al., 2018). Similarly, plant-derived cellulose is broadly considered nontoxic based on a consensus of studies assessing cell cytotoxicity and genotoxicity (Jorfi & Foster, 2015).

Jorfi and Foster (2015) summarized the state of research into the toxicology of nanocellulose-based materials for biomedical applications, as seen in Table 9.1. Overall, the general consensus shows that there is no evidence for serious adverse health effects from nanocellulosic material on the cellular and genetic level, or for in vivo animal experiments (Jorfi & Foster, 2015). These results apply for nanocellulose in aqueously dispersed hydrogel form.

> *"Cellulose and cellulose derivatives pass through the human body safely, and some of the derivatives can be broken down digestive enzymes into natural metabolites in the gastrointestinal tract"* (Jorfi & Foster, 2015)

However, some safety concerns are still being raised regarding pulmonary inflammation with the inhalation and bioaccumulation of large quantities of fine nanocellulose dust (Lin & Dufresne, 2014), as could be formed during the grinding pretreatment or other dry-state mechanical treatment stages. In addition, the presence of chemical cross-linking residues remaining after hydrogel purification, and novel surface functionalized materials, have the potential for adverse biocompatibility effects for nanocellulose hydrogels (Sheikhi et al., 2019; Shojaeiarani et al., 2019).

9.2.3 Challenges for cellulose-based nanohydrogels

9.2.3.1 Spatial and temporal control of drug release

The spatial and temporal control of long-term drug release has been identified as one of the key challenges for nanocellulose hydrogel-drug delivery systems, and for the

TABLE 9.1 Toxicology of nanocellulose-based materials for biomedical applications (Jorfi & Foster, 2015).

Cellulose type	Toxicology experiment	Results	Reference
CNCs	In vitro cytotoxicity test of CNCs isolated from cotton with a 3D triple-cell coculture model of the human epithelial airway barrier	Lower cytotoxicity and (pro)inflammatory response in comparison with multiwalled carbon nanotubes and crocidolite asbestos fibers	(Clift, Foster, & Vanhecke, 2011)
	In vitro cytotoxicity test of CNCs extracted from cotton with a flow cytometry assay	The low concentrations (0.02−100 μg/mL) of CNCs did not show cell death. However, high concentrations (>200 μg/mL) induced cell death and changes in the gene expression of mammalian fibroblasts. The high concentrations (2000 and 5000 μg/mL) of the CNCs affected the expression of stress- and apoptosis-associated molecular markers	(Pereira, Raposo, & Brayner, 2013)
	In vitro cytotoxicity of the CNCs isolated from cotton with a thiazolyl blue tetrazolium bromide (MTT) assay with 3T3 fibroblast cells	CNCs within the concentration range of 100−1000 μg/mL induced minimal decreases in cell viability after 1 day of cell exposure	(Yang, Bakaic, Hoare, & Cranston, 2013)
	In vitro cytotoxicity evaluation of CNCs with nine different cell lines	No cytotoxic effects in the concentration range (0−50 μg/mL) and with an exposure time of 48 h	(Dong, Hirani, & Colacino, 2012)
	In vitro cytotoxicity evaluation of CNCs with L929 cells	Low cytotoxicity of CNCs at low concentrations	(Ni, Zeng, & Wu, 2012)
CNFs	In vitro cytotoxicity test of neat CNFs and modified CNFs with fibroblast 3T3 cells	The neat CNFs did not exert toxic behavior on fibroblast cells. The neat CNFs showed no effect on the cell membrane, mitochondrial activity, or DNA proliferation. The modified CNFs showed toxic behavior and negative effects on cell survival, viability, and proliferation	(Alexandrescu, Syverud, Gatti, & Chinga-Carrasco, 2013)
	In vitro genotoxicity of CNFs with enzyme comet assay	No significant DNA damage	(Hannukainen, Suhonen, Savolainen, & Norppa, 2012)
BNC	In vitro genotoxicity of BNC nanofibers: (1) Salmonella reversion assay, (2) proliferation assay with mouse embryo fibroblasts (3T3) and Chinese hamster ovary cells, and (3) single-cell gel assay (comet assay)	No mutagenic behavior under conditions used, 10%−20% lower proliferation rate in the presence of BNC nanofibers, and BNC nanofibers did not induce DNA damage under the concentrations tested	(Moreira, Silva, & Almeida-Lima, 2009)
	In vitro and in vivo cytotoxicity of BNC in human umbilical vein endothelial cells (with viability and flow cytometric assays) and mouse model	No toxicity in endothelial cells No biochemical differences were observed after 7 days in animal experiments	(Jeong, Lee, & Yang, 2010)
	In vitro and in vivo toxicity of BNC in human umbilical vein endothelial cells and mouse model	BNC did not induce apoptosis and necrosis in endothelial cell and did not stimulate an immune response in endothelial cells and a mouse model	(Kim, Yang, & Park, 2013)

BNC, Bacterial nanocellulose; *CNC*, cellulose nanocrystal; *CNF*, cellulose nanofiber.

Reproduced from Jorfi M., Foster E.J. (2015) Recent advances in nanocellulose for biomedical applications. Journal of Applied Polymer Science 132:1−19. https://doi.org/10.1002/app.41719.

development of pharmaceutical drugs in general (Klemm et al., 2018). Methods to improve the spatial and temporal control of drug delivery is at the forefront of innovative hydrogel design thinking. Some examples of intelligent design include stimuli-responsive hydrogel systems (Section 6), improved administration techniques to implant the hydrogel into the desired location (i.e., injectable hydrogels), and new hydrogel-drug conjugation tools to improve drug conjugation and release (i.e., nanocellulose functionalization chemistry).

9.2.3.2 Drug conjugation

The existing number of therapeutic molecules in the pharmaceutical pipeline is low, and this issue is exacerbated by the fact that an estimated 90% of the developmental pipeline drugs (as of 2010) consist of poorly soluble molecules (Loftsson & Brewster, 2010). Control of drug loading and drug release profiles are especially difficult for these highly lipophilic drugs, as well as high molecular weight drug molecules, due to the incompatibility they pose with respect to the hydrophilic nature of nanocellulose-based hydrogels (Klemm et al., 2018). In addition to the difficult-to-handle nature of these drugs, drug conjugation to the nanocellulose hydrogel can result in reduction or deactivation of the drug's therapeutic activity (Lin & Dufresne, 2014). This has been reported for the physical conjugation of aspirin to the surface of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-oxidized CNF through negative charge interaction on the fiber surface, which promotes the decomposition of this molecule (Carlsson, Hua, Forsgren, & Mihranyan, 2014).

9.2.3.3 Nanocellulose characterization

Quick, accurate and high throughput characterization of nanocellulose into specific product classifications remains a significant challenge for the burgeoning industry (Beck, Walker, & Batchelor, 2019; Chinga-Carrasco, 2013; Davis, Grolman, Karim, & Gilman, 2015a; Desmaisons, Boutonnet, & Rueff, 2017; Kangas, Lahtinen, & Sneck, 2014; Moser, Lindström, & Henriksson, 2015; Nechyporchuk et al., 2016; Qing, Sabo, & Zhu, 2013). Difficulties in the reproducibility of nanocellulose hydrogel production make it difficult to have confidence in the consistency of biomaterial products that are produced from biological material. Improvements in characterization of these materials are required to establish robust quality control systems for consistent production of nanocellulose hydrogels. Standardized heuristics have been developed for nanocellulose classification based on fiber morphology cut-offs (Tappi, 2013), but accurately determining where the nanocellulose product lies within this framework is a significant challenge. Current characterization tools include scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), ultraviolet-visible (UV-Vis) spectroscopy, and rheology, but these methods have a slow turnaround time that limits industrial applicability.

> *"The optimal measurement technique would be fast, low cost, online, and precise (accurate as well as reliable)"* (Davis, Moon, & Ireland, 2015b)

9.2.3.4 Cost

Currently, manufacturing nanocellulose is energy intensive and expensive, both in terms of time and money (Davis et al., 2015b), making the cost of nanocellulose production

for commodity applications prohibitively high (De France et al., 2017). This is especially relevant for BNC, where the production costs of maintaining advanced control over cell cultivation for BNC is a limiting factor for high-scale commercialization of BNC-based biomedical products. Proposed methods to reduce production costs include (1) the use of alternative feedstocks especially plant wastes, (2) novel bioreactor designs, and (3) process automation and scale-up (Klemm et al., 2018).

9.3 Nanocellulose hydrogel-drug delivery systems

9.3.1 Nanocellulose hydrogel forms

The high surface area, hydrophilicity, and high degree of hydroxyl groups on the surface of nanocellulose contribute to their gel formation in aqueous suspension, making it an attractive candidate to produce biobased hydrogels. However, nanocellulose hydrogels can take different forms depending predominantly on the following factors:

- Source material (plant vs. animal vs. bacteria)
- Nanocellulose type (CNC vs. CNF)
- Nanohydrogel form (pure vs. nanocomposite)
- Cross-linking type (physical vs. chemical)

As discussed previously, the nanocellulose source impacts the biochemical composition, fiber properties and network structure. Similarly, the nanocellulose type has a large impact on the hydrogel's mechanical stability and dictates hydrogel form; CNCs are more suited for stabilizing nanocomposite hydrogels due to their lower aspect ratio and mechanical properties. This limits their application to be the reinforcing cross-linking phase in other polymer matrix hydrogels, through either physical or chemical cross-linking. On the other hand, CNF and BNC are typically used to form the matrix backbone of the hydrogel system. Physical self-cross-linking between CNF products with opposing charges occurring to improve mechanical stability of the hydrogel. CNF and BNC can also be incorporated into a polymeric matrix as a reinforcing agent (Sheikhi et al., 2019). Fig. 9.2 provides a general overview of the factors presented above, with a more detailed overview of different nanocellulose hydrogel forms presented elsewhere (Mohammadinejad, Maleki, & Larrañeta, 2019).

9.3.2 Mechanisms of drug loading

Loading of the active therapeutic molecule is an essential step in the hydrogel fabrication process and plays a large role in the controlled delivery of the drug. Drug loading can be achieved through covalent or noncovalent attachment techniques. Convention methods for hydrogel-drug loading include the noncovalent techniques of physical entrapment or postsynthesis loading. Drug entrapment is performed during the hydrogel synthesis process, such that the hydrogel network forms around the drug molecules and prevents their escape through steric hindrance. This class of drug loading includes spraying (Kolakovic, Laaksonen, & Peltonen, 2012a), cross-linking (Lin, Huang, & Chang, 2011; Wang & Chen, 2011), and concurrent drying techniques (Kolakovic, Peltonen, & Laukkanen, 2012b).

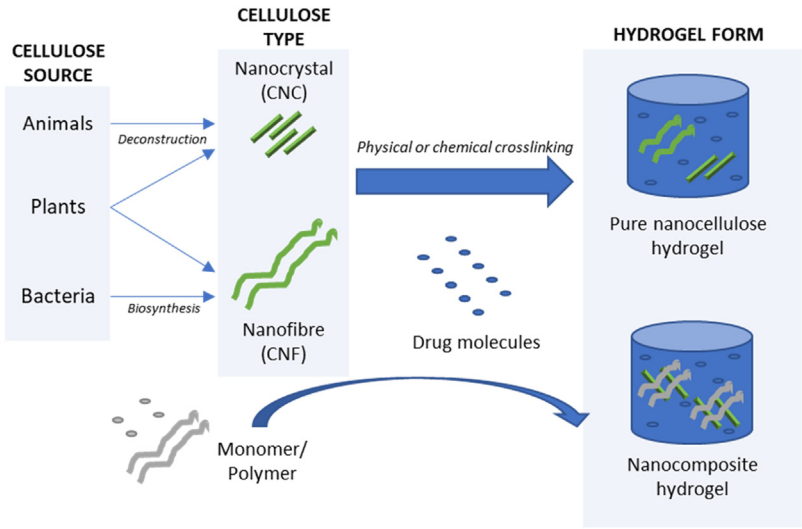

FIGURE 9.2 General schematic outlining the production of nanocellulose hydrogels for controlled drug delivery.

Postsynthesis loading entails the diffusion and adsorption of drug molecules into the already fabricated hydrogel system, which is typically achieved through agitated adsorption, boiling, coating, and soaking techniques. These methods are commonly employed because of their facile nature under relatively mild conditions, with minimal impact on drug stability or efficacy (Klemm et al., 2018). However, a drawback of conventional postsynthesis techniques is their prolonged loading time, which is not applicable for clinical or industrial applications (Sheikhi et al., 2019). To counteract this issue, high speed loading techniques such as vortex-mixing have been developed to achieve loading with as little as 10 minutes (Müller, Wesarg, & Hessler, 2014).

Alternatively, some chemical pretreatment methods such as TEMPO oxidation modify the charge on the surface of nanocellulose fibers, which encourages the physical bonding of charged drug molecules to the nanocellulose matrix (Sheikhi et al., 2019).

Conversely, active loading involves covalent drug conjugation to the nanocellulose fiber surface, which aims to improve control of spatial and temporal drug release and limit the typically undesirable effects of a burst release profile. As mentioned previously, the capacity for nanocellulose to undergo a wide range of chemical surface modification enables covalent conjugation between nanocellulose and drug molecules.

> *"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"* (Sheikhi et al., 2019)

An innovative method of hydrophobic drug conjugation was developed by Valo et al. (Valo, Kovalainen, & Laaksonen, 2011), where a genetic engineering approach was employed to immobilize itraconazole nanoparticles within a nanofibrillar cellulose matrix. Hydrophobin fusion proteins were coupled with cellulose binding domains (CBDs), which

enabled binding to the nanocellulose matrix. The hydrophobin fusion proteins were coated with itraconazole nanoparticles to facilitate drug conjugation, enhancing the storage stability of this drug delivery system up to 10 months.

9.3.3 Mechanisms of drug release

The three main mechanisms that influence the rate of drug release from the nanohydrogel systems are degradation, swelling, and deformation. The dependence between these mechanisms and drug release rate provides a few parameters in which to control drug delivery rate. Firstly, nanocellulose degradation, which is exacerbated by enzymatic or hydrolytic attack, can occur at either the cellulose fiber backbone or at cross-linking point (Shojaeiarani et al., 2019). Factors that influence the rate of nanocellulose degradation include the fiber crystallinity, hydration, surface chemistry triggering immune response. However, animals don't endogenously produce the cellulase enzyme, so nanocellulose degradation is typically slow in normal in vivo conditions (Lin & Dufresne, 2014).

The degree of hydrogel swelling influence the diffusion coefficient for drug molecules within the network. Swelling can occur in response to a range of external stimuli, such as temperature, solvent ionic strength, electrical change, pH, and UV light, and is the predominant mechanism by which stimuli-responsive hydrogel systems operate (Shojaeiarani et al., 2019).

Lastly, deformation of the hydrogel network structure can occur in response to mechanical disturbance, through ultrasound and magnetic field-induced disturbances, or as a result of the foreign body response initiated by immune cells and molecules (Shojaeiarani et al., 2019).

Typically, nanocellulose hydrogels exhibit a biphasic drug release profile, with an initial burst release phase within the first few hours of administration, and a subsequent slow release phase for up to 3 days postadministration of the hydrogel. To counteract these conventional drug release profiles, nanocellulose hydrogels have been functionalized through chemical modification or physical incorporation of components, which strengthen drug binding, increase stability, or prolong drug release and expand the range of applicable drugs (Klemm et al., 2018), as seen in Fig. 9.3.

9.3.4 Scope of book chapter

In the following sections, we aim to outline the range of nanocellulose-containing drug delivery hydrogels published within the literature, including plant-based CNCs and CNFs as the matrix component or cross-linking agent in the hydrogel system. Articles must also involve characterization of drug loading and release mechanisms from hydrogel materials, including stimuli-responsive hydrogel systems. Alternative product forms for drug delivery systems such as tablet excipient and aerogels are considered out of scope, as are non-drug delivery applications of hydrogels and the design and development of drug molecules themselves (Table 9.2).

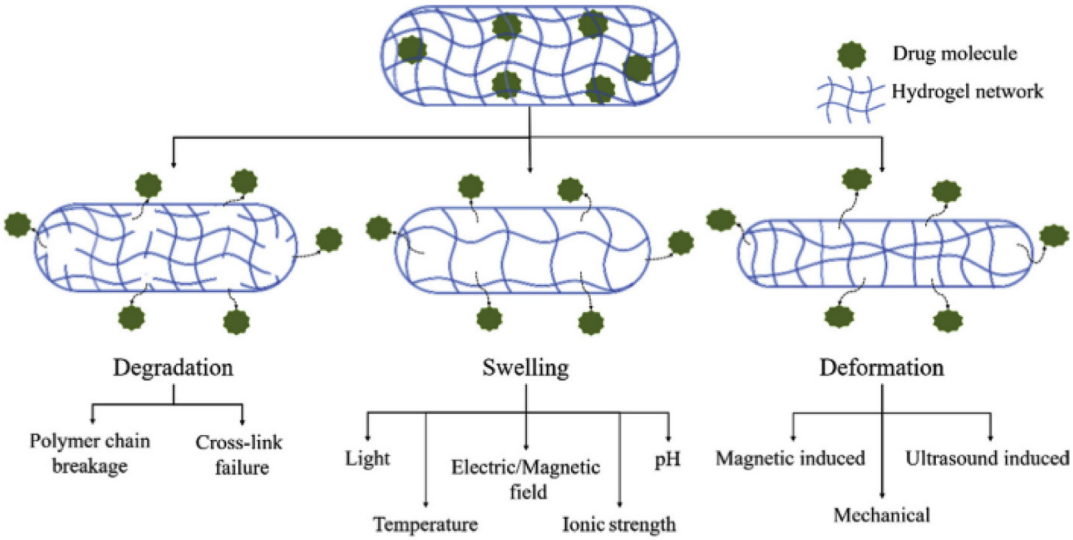

FIGURE 9.3 Mechanisms and triggers of drug release from hydrogel systems (Shojaeiarani et al., 2019). Source: *Reproduced with permission from Carbohydrate Polymers.*

TABLE 9.2 Scope of nanocellulose hydrogel overview.

In scope	Out of scope
• Hydrogels containing nano-scale cellulose • Plant-based CNCs and CNFs as the matrix component OR cross-linking agent in hydrogels • Drug delivery hydrogel systems • Stimuli-responsive drug delivery hydrogels	• Synthetic cellulose forms, that is, cellulose acetate • Micro- or macro-scale cellulose fibers • Bacterial-based CNF hydrogels • Drug-free hydrogel systems • Tissue engineering, biosensor, or biocatalyst applications • Drug development/design

CNC, cellulose nanocrystal; *CNF*, cellulose nanofiber.

9.4 Cellulose nanocrystal hydrogels for controlled drug delivery

9.4.1 Overview of cellulose nanocrystal hydrogels

The inhomogeneous nature and low degree of entanglement of the cellulose network hinders the mechanical properties of the CNCs in pure hydrogel form. Low entanglement is due to the presence of loops, dangling ends, and heterogeneity in cross-link density, which results in lower entrapment of water and other soluble molecules within the CNC network during hydrogel preparation. The result is the formation of a brittle hydrogel due to the rigid structure with a low degree of cross-linking (Shojaeiarani et al., 2019). This leads to CNCs being more applicable as cross-linking agents.

CNCs have an extensive history as nano-reinforcement agents due to their excellent mechanical properties and high potential for surface functionalization. In the case of drug

delivery hydrogels, reinforcement involves providing cross-linking support for the polymeric matrix component. Cross-linking ensures appropriate mechanical properties for the hydrogel to prevent fracture or accelerated biodegradation of the hydrogel, which in turn influences the release rate of the loaded drug. In addition, CNC incorporation reduces the void volume within physically cross-linked hydrogel structures, preventing excessive leakage of the drug from the hydrogel (Shojaeiarani et al., 2019).

9.4.2 Cellulose nanocrystal cross-linked nanocomposite hydrogels

Early work performed by Zhang et al. (Zhang, Huang, & Chang, 2010) incorporated CNCs as cross-linking agents in biobased hydrogels. This project was the first to involve CNCs into a supramolecular hybrid nanocomposite hydrogel system with cyclodextrin (CD), using a polymer inclusion technique. The incorporation of CNCs into the hydrogel accelerated gelation time, enhanced mechanical strength, improved erosion resistance, and allowed long-term sustained release of the loaded drug—in this case bovine serum albumin (BSA) as the model macromolecule. Similarly, Wang and Chen (Wang & Chen, 2011) used CNCs as a physical cross-linking agent to produce an all-cellulose hydrogel system that exhibited controlled release of BSA. The matrix component in this case was regenerated cellulose. The CNCs functioned as a molecular bridge between cellulose fibers during the gel formation process.

Lin et al. also demonstrated the capability of CNCs to cross-link a network of sodium alginate (SA) fibers. In this work, microspheres of CNC-filled SA showed more consistent swelling patterns and higher encapsulation efficiency, as well as a sustained release profile of the respiratory disease drug theophylline. The mechanisms behind the effective controlled drug delivery of this system are thought to be due to restricting the motion of the matrix polymer (i.e., SA chains), inhibiting the diffusion of theophylline through the polymeric network, and slowing the degradation of the microspheres (Lin et al., 2011).

A less common technique is chemical cross-linking of hydrogels using surface-modified CNCs, due to the additional steps required in the hydrogel fabrication process. Mauricio et al. (Mauricio, Da Costa, & Haraguchi, 2015) introduced vinyl groups to both CNCs and the starch matrix component to produce a covalently bound hydrogel composite. The release rate of vitamin B12 as the model drug became 2.9 times slower with the addition of CNCs to the starch hydrogel, signifying the retardant function of nanocellulose on hydrogel-drug release.

Lin and Dufresne (Lin & Dufresne, 2013) used surface modification to produce a chemically grafted CNC-based hydrogel with cyclodextrin and Pluronic polymers consisting of PPG and PEG. Loading of the cancer drug doxorubicin into the rigid, chemically cross-linked CNC hydrogel presented extended drug release with proposed "*nano-obstruction*" and "*nanolocking*" mechanisms.

Lin et al. (Lin, Gèze, & Wouessidjewe, 2016) described the fabrication of novel double-membrane hydrogel-drug carrier by employing electrostatic interactions between cationic cellulose nanocrystals and anionic alginate. The inner microsphere hydrogel of CNCs and SA was produced through drop-wise addition of the composite suspension with a syringe into a Ca^{2+} cross-linking solution. The outer membrane hydrogel was produced by subsequently

dropping these microspheres into a 1.5 wt.% SA solution. The double-membrane structure allowed the encapsulation and codelivery of two drugs into the different layers for rapid drug release in the outer layer, and sustained release from the inner layer.

You et al. (You, Cao, & Zhao, 2016) demonstrated an injectable hydrogel based on cationic CNCs and quaternized cellulose (QC), stabilized through cross-linking with β-glycerophosphate. The strong interaction between CCNCs and QC led to improvement in the mechanical strength and dimensional stability of the hydrogel. In addition, the hydrogel exhibited sustained release of doxorubicin (DOX), which was injected into the tumor site of cancer-bearing mice, showing no obvious cytotoxicity and inflammatory reactions and good in vivo anticancer efficacy.

The incorporation of CNCs into a chitosan-based hydrogel matrix is one of the most common bionanocomposite hydrogel systems for controlled drug delivery (Sheikhi et al., 2019). Sampath et al. showed that the incorporation of up to 2.5% CNCs in a chitosan hydrogel matrix created a stable hydrogel with a high cross-linking degree of 83.6%, improved the maximum compression of the hydrogels from 25.9 ± 1 to 50.8 ± 3 kPa, and exhibited excellent pH sensitivity, with a maximum swelling ratio of 222 ± 10% at pH 4.01 (Sampath, Ching, & Chuah, 2017).

9.4.3 Physically cross-linked cellulose nanocrystal hydrogels

The mechanisms for physical CNC cross-linking involve the stabilization of the matrix network through enhancing secondary force bonding. Physical cross-linking has advantages over chemical cross-linking in the lack of toxic cross-linking agents, higher biodegradability, reversible properties, shear thinning behavior, and self-healing capabilities (Shojaeiarani et al., 2019). Despite the advantages, physically cross-linked CNC hydrogels don't have robust control over the degradation rate in situ, confining their use to short-acting drug release systems (Cheng, He, & Ren, 2018).

9.4.4 Chemically cross-linked cellulose nanocrystal hydrogels

The mechanism of chemical cross-linking aims to enable CNCs to be the hydrogel matrix component, through the incorporation of a reactive functional component that can initiate covalent bonding of the polymeric cellulose crystals. The advantages of this system are that chemical cross-linking generates more uniform properties and less swelling sensitivity compared to physical cross-linking. This leads chemically cross-linked hydrogels to typically have better control over the drug release profile compared to physical cross-linked hydrogels. However, their use as biomedical products is limited due to the residual presence of potentially toxic initiator and cross-linker chemicals that are difficult to remove, even through hydrogel purification (Shojaeiarani et al., 2019).

9.4.5 List of cellulose nanocrystal hydrogels

Table 9.3 details the published work investigating CNC hydrogels for controlled drug delivery applications.

TABLE 9.3 Cellulose nanocrystal (CNC) hydrogels as controlled drug delivery systems.

CNC role	Matrix component	Cross-linking agent(s)	Drug type	Key points	Application	Citation
Physical cross-linking agent	• PEO_{137}-b-PPO_{44}-b-PEO_{137} (EPE) • α-cyclodextrin (α-CD)	CNCs	Bovine serum albumin (BSA)	• Hydrogel elastic modulus increased by 50 times over native hydrogel due to CNC reinforcement • Incorporation of CNCs presented accelerated gelation, enhanced mechanical strength, improved erosion resistance of solution, and long-term sustained release of drugs, and no additional cytotoxicity due to inclusion of CNCs	Injectable, controlled drug delivery system	(Zhang et al., 2010)
Physical cross-linking agent	Alginate-based microspheres	• CNCs • Ca^{2+}	Theophylline	• Introduction of CNCs improved the mechanical performance and crystalline properties of nanocomposite microspheres, which showed more consistent swelling patterns, higher encapsulation efficiency, and sustained release profiles of the drug than native microspheres • Good dispersion of CNCs in microspheres could restrict the motion of the SA polymer chains, inhibit diffusion of the drug, and slow the dissolution and collapse of microspheres, improving drug loading and release behavior	Controlled drug delivery system	(Lin et al., 2011)
Physical cross-linking agent	• Chitosan (CS) • Xanthan (XG)	CNCs	5-Flurouracil (5-FU)	• CNCs encourage hydrogen bonding and complexation with polymer chains, leading to improved mechanical performance, good biocompatibility and controlled release of 5-FU • Mechanical performance of BNC hydrogels significantly improved with the incorporation of 2–10 wt.% content of CNCs	Anticancer drug delivery system	(Madhusudana Rao, Kumar, & Han, 2017)

(*Continued*)

TABLE 9.3 (Continued)

CNC role	Matrix component	Cross-linking agent(s)	Drug type	Key points	Application	Citation
Physical cross-linking agent	Regenerated cellulose	CNCs	Bovine serum albumin (BSA)	• CNCs could act as the "bridge" to facilitate the cross-linking of cellulose chains during gel formation, improving their dimensional stability and mechanical strength • Composite CNC gels exhibited dense surfaces and well-organized porous internal structure • Composite gels demonstrated capacity to steadily release BSA in simulated body fluid	Controlled drug delivery system	(Wang & Chen, 2011)
Physical cross-linking agent	Gelatin	• CNCs • Glutaraldehyde	Theophylline	• CNC-reinforced gelatin hydrogels formed semiinterpenetrating polymer networks (semiIPNs) • CNC-gelatin hydrogels showed excellent pH sensitivity, with a maximum swelling ratio at pH 3 • The amount of chemical cross-linking agent could be reduced for the same mechanical strength with the incorporation of CNCs	Controlled pH-responsive drug delivery system	(Ooi, Ahmad, & Amin, 2016)
Physical cross-linking agent	Anionic sodium alginate (SA)	• Cationic cellulose nanocrystals (CCNC) • Ca^{2+}	• Ceftazidime hydrate (CH) • Epidermal growth factor (EGF)	• The double-membrane hydrogel—which involves an external membrane composed of neat alginate and an internal composite hydrogel composed of cationic CNC and anionic alginate—ensured codelivery of the drug complex • Rapid release for the shell-loaded antibiotic (Ceftazidime hydrate) was achieved in vitro within the first 3 days, and then sustained release of the core-loaded epidermal growth factor (EGF) was achieved from day 4 to day 12 • The presence of rigid CNCs within the inner membrane of the hydrogel structure enhanced the stability—and therefore sustained drug release—due to electrostatic interactions between cationic nanocrystals and anionic alginate	Controlled drug codelivery system	(Lin et al., 2016)

Physical cross-linking agent	Alginate	• Calcium chloride • Honey	Rifampicin	• Rifampicin-loaded alginate cellulose nanocrystal hybrid nanoparticles were synthesized by ionic gelation, and subsequently reduced to a particle size of 100 nm by probe sonication to avoid renal clearance and promote cellular uptake • The drug release profile of just 15% rifampicin after 2 hours demonstrated the capability of this hydrogel system to provide sustained release under low pH gastric conditions, indicating a better outcome for the treatment of *Mycobacterium tuberculosis*	Controlled drug delivery system	(Thomas, Latha, & Thomas, 2018)
Physical cross-linking agent	Chitosan	• Oxidized CNCs • Pentasodium tripolyphosphate (TPP)	Repaglinide (RPG)	• Cellulose nanocrystal (CNC) and chitosan nanoparticle (CHNP) was loaded with Repaglinide through ionic gelation, yielding a drug encapsulation efficiency of ~98%. Subsequently, oxidation introduced carboxylic groups to the CNC surfaces (OXCNC), further slowing Repaglinide release rate • This system proved suitable for antidiabetic controlled-release drug delivery	Controlled drug delivery system	(Abo-Elseoud, Hassan, & Sabaa, 2018)
Physical cross-linking agent	Polyvinyl alcohol (PVA)	CNCs	Fluorescein isothiocyanate isomer I (FITC)	• The incorporation of CNCs into the PVA matrix exhibited extended controlled release of the fluorescent marker (FITC) from the contact lens structure in the presence of a physiological lysozyme concentration (0.2 mM) • The hydrogel system shows promise for the development of controlled ophthalmic drug delivery systems	Controlled drug delivery system	(Åhlén, Tummala, & Mihranyan, 2018)
Chemical cross-linking agent	Vinylated starch	Vinylated CNCs	Vitamin B12	• The introduction of vinyl bonds to both CNCs and starch created a microhydrogel composite in which CNCs played a role as a covalent cross-linking agent • CNCs acted as a retardant factor for drug release, with vitamin B12 release rate becoming 2.9 times slower with their addition	Controlled drug delivery system	(Mauricio et al., 2015)

(*Continued*)

TABLE 9.3 (Continued)

CNC role	Matrix component	Cross-linking agent(s)	Drug type	Key points	Application	Citation
Chemical cross-linking agent	Pluronic polymers	• β-cyclodextrin grafted CNCs • α-cyclodextrin	Doxorubicin hydrochloride (Dox · HCl)	• Supramolecular hydrogels were constructed through the semichemical immobilizisation of Pluronic polymers on the surface of β-cyclodextrin grafted CNCs. α-cyclodextrin induced in situ hydrogel formation due to "host − guest" inclusion of uncovered PEG polymer chains • This supramolecular structure showed an enhanced level of CNC loading, resulting in increased structural and thermal stability and a more gradual drug release profile than controls without CNCs • This hydrogel system exhibited prolonged drug release of DOX.HCl, with special release kinetics due to the "obstruction" and "locking" effects • The reversible thixotropic behavior of this hydrogel system makes it suitable injectable drug delivery applications	Injectable, controlled drug delivery system	(Lin & Dufresne, 2013)
Chemical cross-linking agent	Quaternized cellulose (QC)	• Cationic CNCs • β-glycerophosphate	Doxorubicin (Dox)	• The hydrogel system was formed through in situ thermo-gelling between Quaternized cellulose (QC) and Cationic CNCs, mediated by the cross-linking agent β-glycerophosphate • The strong interaction between QC and CCNCs resulted in an orders-of-magnitude increase in mechanical strength (∼200-fold increase in storage modulus over pure QC/β-GP hydrogels), an extension of in vitro degradation time, and improved in vivo sustained drug release of DOX when injected adjacent to tumors within mice bearing liver cancer xenografts	Injectable, controlled drug delivery system	(You et al., 2016)

Hydrogel matrix + chemical cross-linking agent	• Chitosan (CS) • Dialdehyde CNCs (DACNCs)	Dialdehyde CNCs (DACNCs)	Theophylline	• Chitosan was cross-linked using DACNC as both the matrix and cross-linker in different weight ratios • Cumulative drug release of the CNC/CS3 hydrogel was ~85% and 23% in the gastric (pH 1.5) and intestinal (pH 7.4) fluids, respectively	Gastric-specific drug delivery system	(Xu, Ji, & Sun, 2019)
Hydrogel matrix	TEMPO-oxidized CNCs	• Ca^{2+} • Al^{3+}	Clofazimine (CFZ)	• Sustained release of a large amount—up to 37% w/w—of the lipophilic drug clofazimine from nanocellulose hydrogels • Di- or trivalent cations were used as gelling agents to prepare hydrogels with different stiffness, obtaining rate-controlled DDSs • Surfactant coloading is a successful strategy to increase drug solubility in water by about 50 times, while avoiding initial burst release	Controlled lipophilic drug delivery system	(Piotto & Bettotti, 2019)

9.5 Cellulose nanofiber-based hydrogels for controlled drug delivery

9.5.1 Overview of cellulose nanofiber hydrogels

Native CNF without modification are amphiphilic materials, with both hydrophilic and hydrophobic sections that are capable of adsorbing and enabling the controlled release of hydrophobic drugs (Sheikhi et al., 2019). CNF can be loaded with drugs through swelling in a drug suspension, where drug molecules are physically entrapped within the nanocellulose network structure.

Readily injectable CNF hydrogels may have application as surgical adhesives, space-filling biomaterials or tissue engineering substrates, applicable for parenteral (implants), topical (transdermal patches), or ocular applications (Kolakovic et al., 2012b; Laurén, Lou, & Raki, 2014).

9.5.2 Cellulose nanofiber hydrogels

Valo et al. (Valo et al., 2011) developed a nanodispersion system by immobilizing itraconazole nanoparticles within a nanofibrillar cellulose matrix, to enhance the drug's processing and storage stability. A genetic engineering approach was employed to couple hydrophobin fusion proteins with CBDs, which coated the surface of itraconazole nanoparticles and enabled binding to the nanocellulose matrix. In addition to enhanced stability of up to 10 months, the release rate of itraconazole from the nanocellulose suspension was slightly attenuated.

Kolakovic et al. (Kolakovic et al., 2012b) were able to encapsulate water-insoluble drugs, including indomethacin, itraconazole, and beclomethasone, into a cellulose nanofiber network through hydrogel preparation and filtration to form a tight fiber network film. The entrapment efficacy of hydrophobic drugs in the dried hydrogel film was $> 90\%$, exhibited mechanical properties suitable for easy handling, and sustained close to zero-order kinetics for a three-month period.

In addition, Kolakovic et al. (Kolakovic, Peltonen, & Laukkanen, 2013) published an extension of their previous 2012 publication, evaluating the interactions of the same three model drugs and the cellulose nanofiber matrix. Permeation studies found that the drug's structural characteristics (i.e., size) affected the rate of diffusion through the nanocellulose film.

Similarly, Laurén et al. (Laurén et al., 2014) investigated the effect of molecule size on drug release rate from an injectable wood pulp-based cellulose nanofiber hydrogel. Technetium-99m radio-labeling allowed for in vivo imaging of the nanocellulose hydrogel after injection into mice. Drug release from the hydrogel was investigated using a small (123I-β-CIT) and large protein drug (99 mTc-HSA) to evaluate the effect of molecular size on the rate of drug release using pharmacokinetic models in conjunction with technetium-99m labeling. The hydrogel-drug delivery system resulted in a twofold decrease in the in vivo drug elimination rate for the large drug—technetium-99m-labeled human serum albumin—but had no impact on the release rate of the small drug ^{123}I-β-CIT.

To address this issue, Paukkonen et al. (Paukkonen, Kunnari, & Laurén, 2017) developed anionic cellulose nanofiber hydrogels capable of controlling the release rate of small drug molecules with a molecular weight less than 500 M—including metronidazole, nadolol, and ketoprofen—to a moderate extent. In addition, this study determined that freeze-drying nanocellulose hydrogels into aerogels and reconstruction back to into hydrogel form did not significantly impact their drug release properties.

9.5.3 List of cellulose nanofiber hydrogels

Table 9.4 details the published work investigating CNF hydrogels for controlled drug delivery applications.

9.6 Stimuli-responsive nanocellulose hydrogels

9.6.1 Overview of stimuli-responsive hydrogels

The next iteration or hydrogel innovation involves the development of stimuli-responsive, or smart nanohydrogel materials. In the biomedical context, smart hydrogels can function as semitargeted on-demand drug delivery systems, as well as microenvironmentally-responsive tissue engineering materials or intimate biosensor devices.

Smart hydrogels have the capability of changing their degree of swelling, network structure, drug release profile, and mechanical strength in response to stimuli that typically include changes in pH, temperature, electric and magnetic field, light, pressure, and ultrasonic wave frequency (Shi, Zheng, & Wang, 2014).

The stimuli-responsive capabilities of these hydrogels are particularly important for hydrogel-drug delivery systems, as it renders these materials capable of coupling changes in internal environmental conditions within the body with a higher or lower rate of drug release. For example, drugs that sensitize the gastric lining of the stomach, or must otherwise be carried part-way through the digestive tract before being released, can be packaged within a hydrogel matrix that limits drug diffusion and release under low pH conditions. Conversely, hydrogels with an anticancer drug payload/cargo could be designed to sense the lower pH around the tumor site and increase its drug release rate in response (Fig. 9.4).

On the other hand, nanohydrogels engineered with responsiveness to electric field, magnetic field, or near infrared (NIR) light have the capability of on-demand drug release at a specific time, or when the hydrogel has reached a specific location within the body.

9.6.2 Stimuli-responsive nanocellulose hydrogels

Ionizable polymers, including sodium alginate (SA), poly (acrylic acid) (PAA), poly(N-methacryloyl glycine), gelatin, and carboxymethylcellulose, have been utilized as hydrogel components that are responsive to different pH conditions within the human body (Klemm et al., 2018). In early work involving pH-responsive nanocellulose hydrogels, Shi et al. (Shi et al., 2014) incorporated bacterial nanocellulose (BNC) with SA, loading this system with Ibuprofen (IBU) to study the release rate of this model drug under different conditions. SA, with a higher number of ionizable −COO groups, acts as a pH-responsive component in the system. However, due to the limited mechanical performance and rapid drug release profile of SA, nanocellulose is required to stabilize the system. The integration of these two components generated a semiinterpenetrating polymer network (IPN) that improved the mechanical performance of this hydrogel system.

In addition, the incorporation of BNC into SA generated a higher specific surface area for the internal porous microstructure, improving drug solution uptake, but also importantly increasing the number of active −COO− groups within the microstructure, resulting

TABLE 9.4 Cellulose nanofiber (CNF) hydrogels as controlled drug delivery systems.

Matrix component	Cross-linking Agent(s)	Drug type	Key Points	Application	Citation
Technetium-99m labeled CNF	-	• Small drug (123I-β-CIT) • Large drug (99mTc-HSA)	• The radiolabeled CNF allows for tracing the in vivo localization of the hydrogel • Testing the effect of molecule size on drug release rate showed that the CNF hydrogel decreased elimination rate of the large drug (99mTc-HAS) twofold but did not change the release rate of a small drug (123I-b-CIT)	Injectable controlled drug delivery system	(Laurén et al., 2014)
CNF film	-	• Indomethacin • Itraconazole • Beclomethasone	• A hydrogel film was produced, with entrapment of up to 40% loading for the hydrophobic drugs achieved through a simple filtration method • Sustained drug release over a period of three months • Drug release kinetics are dependent on the specific drug due to different levels of drug solubility	Controlled drug delivery system	(Kolakovic et al., 2012b)
Anionic nanofibrillar cellulose (ANFC)	• Ca^{2+} • Al^{3+} • Fe^{3+}	• Metronidazole (MZ) • Nadolol (NAD) • Ketoprofen (KETO) • FITC-dextran (FITC-DEX) • Lysozyme (LZ) • Bovine Serum Albumin (BSA)	• Freeze-drying did not affect the drug release properties from redispersed ANFC hydrogels, indicating this system could be stored in dry form and only redispersed when required • For large drug-like molecules, the ANFC hydrogel with a higher fiber content resulted in significantly lower drug diffusion coefficients, whereas only moderate control was achieved over the release of small molecules	Controlled drug delivery system	(Paukkonen et al., 2017)

Deacetylated α-chitin nanofibers (α-DECHN) (TEMPO)-oxidized CNF (TOCNF)	-	5-fluorouracil (5-FU)	• Physical cross-linking was achieved through the interaction between cationic α-DECHN and anionic TOCNF, with a higher degree of cross-linking resulting in a higher drug-loading efficiency and drug release percentage • The composite hydrogel with 40% α-DECHN and 60% TOCNF had the strongest cross-linked structure and largest swelling ratio	Controlled drug delivery system	(Xu, Liu, & Chen, 2018)
Nanofibrillated 2,3-dicarboxyl cellulose (nf-DCC)	-	Piroxicam (PRX)	• The extent of drug loading is governed by surface charge density and carboxylate group content • Prolonged release of PRX over several hours was observed upon exposure of the loaded membranes to simulated human skin fluid	Controlled transdermal drug delivery system	(Plappert, Liebner, Konnerth, & Nedelec, 2019)
CNF	Ca^{2+}	• Bovine serum albumin (BSA) • Fibrinogen • Lysozyme	• Studying the loading and release profile of three model drugs of varying size and isoelectric point revealed that negatively charged compounds had size-dependent release profiles—larger fibrinogen release was five times lower than its smaller version • Electrostatic interactions between the proteins and the negatively charged NFC hydrogel played a central role in drug loading and release profiles	Controlled drug delivery system	(Basu, Strømme, & Ferraz, 2018)

(*Continued*)

TABLE 9.4 (Continued)

Matrix component	Cross-linking Agent(s)	Drug type	Key Points	Application	Citation
CNF	• Xyloglucan • Pectin	• 70 kDa FITC-dextran • 2000 kDa FITC-dextran	• The stimuli-responsive (ON/OFF) permeability of the capsule wall, which is exclusively made dietary fiber components such as CNF, xyloglucan, and pectin, allows for trapping of drug compounds under saline conditions and released when in water. • This biomimetic system could be applied as a colon targeted delivery vehicle in the gastrointestinal tract	Controlled drug delivery system	(Paulraj, Riazanova, & Svagan, 2018)
Metformin surface-modified CNF	-	Metformin	• Metformin was successfully attached on the surface of cellulose nanofibers through electrostatic interaction, making Met-Cel-NFs gels a potentially effective system for the prevention of melanoma cancer metastasis • Met-Cel-Nfs gel could be easily sterilized and injected • Met-Cel-NFs gels mimicked the viscoelastic properties of the extracellular matrix around the tumor site	Controlled anticancer drug delivery system	(Nurani, Akbari, & Taheri, 2017)
DOX surface-modified CNF	-	Doxorubicin (Dox)	• DOX surface-modified CNF hydrogels showed suitable injectability, a sustained drug release profile, enhanced in vitro cytotoxicity against melanoma, and blocked the migration of melanoma cells from the tumor site	Controlled anticancer drug delivery system	(Alizadeh, Akbari, Nurani, & Taheri, 2018)

CNF	Genetically engineered hydrophobin fusion protein	Itraconazole	• A genetic engineering approach was employed to immobilize itraconazole nanoparticles within a nanofibrillar cellulose matrix, using hydrophobin fusion proteins coupled with cellulose binding domains (CBDs) • Hydrophobin fusion proteins were coated with itraconazole nanoparticles to facilitate drug conjugation, enhancing the storage stability of this drug delivery system to up to 10 months The nanofibrillar matrix provides protection for the nanoparticles during the formulation process and storage and slightly attenuating the release rate of itraconazole	Controlled drug delivery system	(Valo et al., 2011)

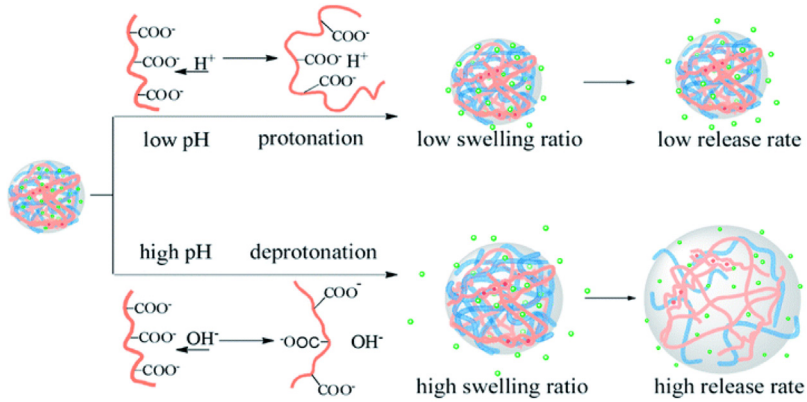

FIGURE 9.4 pH-responsive behavior of a bacterial nanocellulose (BNC) + sodium alginate (SA) semiinterpenetrating polymer network hydrogel developed by Shi et al. (2014). Source*: Reproduced with permission from RSC Advances.*

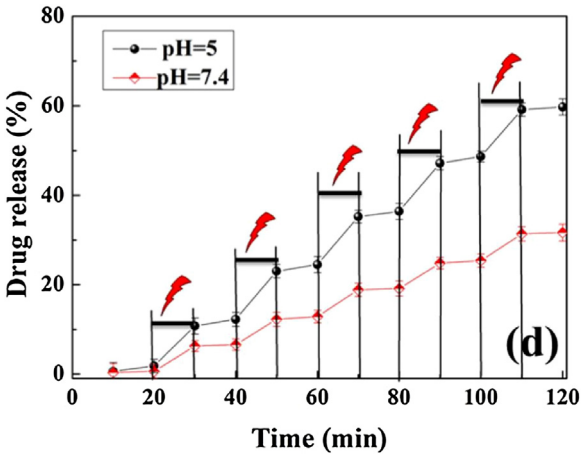

FIGURE 9.5 Stepwise drug release with the application of NIR irradiation to a TEMPO CNF + PDA hydrogel loaded with the antibiotic drug tetracycline hydrochloride (Liu et al., 2018). Source*: Reproduced with permission from Carbohydrate Polymers.*

in a hydrogel with a higher sensitivity to pH (and electric) stimuli. The controlled release of IBU, an important drug to treat musculoskeletal and joint disorders, is motivated from the need to limit irritation of the gastrointestinal mucosa, which warrants colonic targeting of the drug. The IBU release rate was faster under alkaline conditions, in contrast to the acidic conditions of the stomach. The release rate could also be further enhanced through the application of an electric field (Shi et al., 2014).

Another example of an on-demand nanocellulose hydrogel system was developed by Liu et al. (Liu, Sui, & Liu, 2018), incorporating photothermal polydopamine (PDA) into a TEMPO-oxidized CNF hydrogel network. PDA has previously been found to quickly and efficiently convert NIR irradiation into heat, which results in an increase in drug release from the hydrogel (Fig. 9.5).

In addition to the NIR responsivity of PDA, it also consists of a number of functional groups, such as catechol and quinone, that act as attachment sites for the incorporation of drug molecules into the hydrogel system, or as cross-linking sites with CNF to improve the mechanical performance of the hydrogel. However, a limitation of this system is the

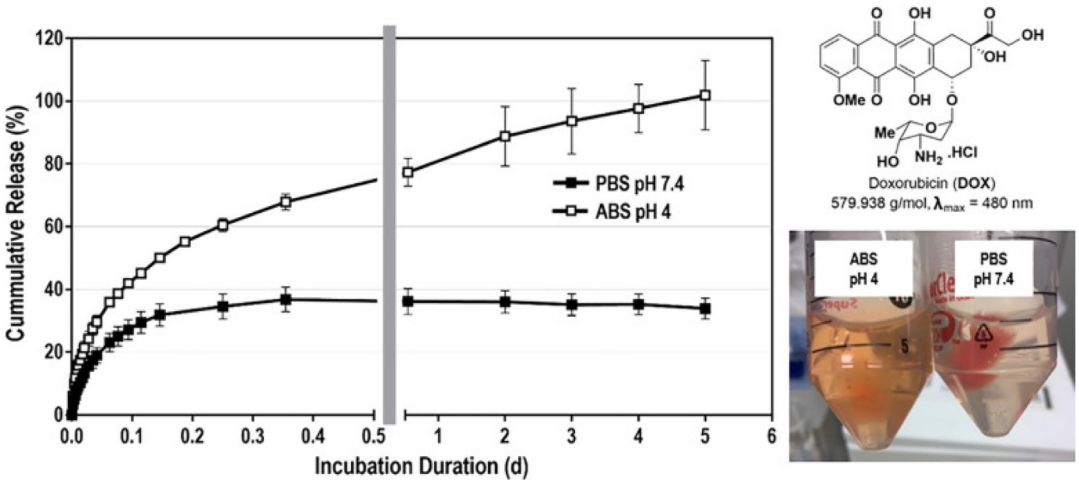

FIGURE 9.6 Release rate of DOX from the (DCC180 + CDAC180)Ac4 hydrogel under two different pH conditions (Hujaya et al., 2018). Source: *Reproduced with permission from Acta Biomaterialia.*

low maximum drug-loading capacity of 14.4%, which creates a challenge for effectively scaling up this material into a biomedical product.

A hydrogel system not hindered by encapsulation efficiency was developed by Hujaya et al. (Hujaya, Lorite, Vainio, & Liimatainen, 2018). This system involves the incorporation of two chemically modified CNFs with opposing charges in order to form a polyion complex hydrogel. Both cellulose components initially underwent mechanical treatment and periodate oxidation for either 30, 60 or 180 minutes, before being separated and treated with either sodium chlorite to produce anionic CNF containing carboxylic acid groups (DCC), or with Girard's reagent T to produce cationic CNF containing quaternary ammonium groups (CDAC). The hydrogel was formed and drug loaded through dissolving the anticancer drug DOX into DCC before mixing with CDAC. The gelation process to generate the polyion complex hydrogel entrapped DOX within the CNF matrix and resulting in 100% loading efficiency within the hydrogel. This feature would allow reliable patient-specific dosage for the hydrogel-drug formulation.

This study was the first exhibition of a complementary CNF polyion complex hydrogel, with the (DCC180 + CDAC180) hydrogel at pH 4–5 showing an approximate 10-fold increase in storage and loss moduli compared to the individual dispersions. Considering pH-dependent drug release, the (DCC180 + CDAC180) hydrogel showed a slower release rate with a lower cumulative release at physiological pH (pH = 7.4), whereas at pH 4 the hydrogel exhibited a faster burst release phase followed by a sustained release phase that achieved complete DOX release after 5 days. This feature could allow higher release of DOX at pathophysiological tumor sites, known to be slightly more acidic relative to normal physiological tissue. However, this system is not readily injectable due to the robust ionic cross-linking generated between the anionic and cationic CNF components during hydrogel fabrication (Hujaya et al., 2018) (Fig. 9.6).

9.6.3 List of stimuli-responsive nanocellulose hydrogels

Table 9.5 details the published work investigating stimuli-responsive nanocellulose hydrogels for controlled drug delivery applications.

TABLE 9.5 Stimuli-responsive nanocellulose hydrogel-drug delivery systems.

Matrix component	Cross-linking agent	Response stimuli	Drug type	Key points	Citation
• Bacterial cellulose (BNC) • Sodium alginate (SA)	$CaCl_2$	• pH • Electrical	Ibuprofen	• From pH 1.5 to 11.8, the swelling ratio increased from <8 times to >13 times compared to its dry weight • For electric field from0 to 0.5 V, hydrogels increasing swelling ratio from 8 to 14 times	(Shi et al., 2014)
• Magnetic-CNCs • Sodium alginate	Ca^{2+}	pH	Ibuprofen	• pH-sensitive based hydrogel beads composed of magnetic nanocellulose and sodium alginate as a drug carrier for Ibuprofen • Studied the effect of magnetic-cellulose nanocrystals on the physical properties of the hydrogel beads, drug loading, encapsulation efficiencies and drug release behavior	(Supramaniam, Adnan, Mohd Kaus, & Bushra, 2018)
• TEMPO CNF • PNIPAAm	-	• pH • Temperature	Methylene blue	• As the carboxylate content increases, the dual responsiveness of hydrogel improved at the expense of the compression strength. At low carboxylate content, FESEM analysis suggested the formation of a honeycomb structure, while high carboxylate content produced large pores	(Masruchin, Park, & Causin, 2018)
• Sodium alginate (SA) • TEMPO CNF	$CaCl_2$	pH	Probiotics	• pH-responsive gel macrospheres can provide efficient protection for probiotics in simulated gastric fluids and targeted release them in simulated intestinal fluids	(Zhang, Yang, & Zhou, 2018)
• Cellulose fiber (CF) • TEMPO CNF	-	pH	Probiotics	• A composite hydrogel material of CF and TEMPO CNF were extruded into macrogels with an extrusion dropping technique • In the composite macrogels, the carboxyl groups in CNF worked as a pore size regulator, whereas the cellulose backbone strengthening the porous structure	(Luan, Zhou, & Zhang, 2018)
• Anionic CNF (DCC) • Cationic CNF (CDAC)	-	pH	Doxorubicin (Dox)	• The polyion complex was formed for the first time between two chemically modified CNFs with opposing charges • The CNF polyion complex hydrogel showing an approximate 10-fold increase in storage and loss moduli compared to the individual dispersions at pH 4–5 • At physiological pH 7.4, approximately 65% of doxorubicin was retained past a burst release regime, while complete release was observed within 5 days at pH 4	(Hujaya et al., 2018)

• PNIPAAm • CNCs	-	Temperature	Metronidazole	• PNIPAAm-CNC hybrid hydrogels exhibited clear thermo-responsive behavior; the volume phase transition temperature (VPTT) was in the range of 36 to 39°C • The hydrogels showed a good drug-loading capacity at room temperature and a burst drug release, which was followed by slow and sustained release at 37°C	(Zubik, Singhsa, & Wang, 2017)
• PAA • CNCs	MBA (N,N-ethylenebisacrylamide)	pH	Theophylline	• Preparation of a pH-responsive hydrogel in the form of a semiinterpenetrating polymer network (IPN) by cross-linking acrylic acid monomers in a CNC suspension • The hydrogel reached maximum swelling at pH 7, with the 15% CNC/PAA hydrogel showed the potential to be used as drug carrier system	(Lim, Rosli, & Ahmad, 2017)
• PVA • CNF	Glutaraldehyde (GA)	pH	Cisplatin	• The PVA hydrogel containing 1 wt.% CNFs effectively slowed the drug release rate, reducing side effects of cisplatin such as nephrotoxicity • This hydrogel system is suitable for the controlled delivery of cisplatin within the slightly basic environment of the small intestine	(Azhar, Shahbazpour, & Olad, 2017)
• Polydopamine (PDA) • TEMPO CNFs	Ca^{2+}	• pH • Near-IR (NIR) light	Tetracycline hydrochloride (TH)	• The hydrogel system composed of PDA and TEMPO CNF showed controlled drug release in response to pH and NIR light • Benefits of this system include (1) easy preparation; (2) multiresponse properties (i.e., pH, NIR response, and long period drug releasing); and (3) excellent wound healing ability—a synergistic effect on promoting wound healing	(Liu et al., 2018)

9.7 Conclusion and future perspectives

Nanocellulose is proving to be a very promising material for high value applications in biomedicine, and in the form of nanohydrogels for controlled drug delivery specifically. This chapter has provided an overview of this emerging class of biomaterial, with a focus on the current research status of CNC and CNF drug delivery hydrogels, and examples of advanced, stimuli-responsive nanocellulose hydrogels published in the literature.

Nanocellulose, both in the form of CNC and CNF, are employed as either the matrix component or cross-linking agents within the hydrogel system. Therapeutic drugs can be loaded into the hydrogel through a range of techniques, which include both passive physical entrapment or adsorption techniques, or active drug conjugation through covalent bonding to the nanocellulose surface. In general, the incorporation of nanocellulose has shown improvements in the mechanical stability of the hydrogel, the release rate profile of the drug, and the application areas for hydrogel systems to include difficult-to-handle drugs and additional material functionalities.

Researchers within this overlapping fields of nanocellulose and biomedicine are exciting by the potential that nanocellulose-based hydrogels hold for smart drug delivery systems but are aware that this field is just passing its infancy and that many challenges still lie ahead. The following categories have been identified as important opportunities, challenges and considerations for the future development of this field.

Nanocellulose Advantages: Cellulose as a resource is abundant, renewable, and produced through a natural biosynthetic process, which provides sustainability benefits over petrochemically derived materials. In addition, its tunable properties and surface functionalization capacity make it a highly flexible for different product forms and application types. Lastly, its predominantly benign safety profile is a boon for its use as a medical biomaterial.

> *"Nanocellulose-based cargo delivery systems can 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."* (Sheikhi et al., 2019)

Variability: A downside to nanocellulose's renewability and tunability is that fabricated products can present high variability, either from natural variation associated with utilizing biomass or batch to batch variation. Consequently, the reliability and reproducibility of production techniques is an essential factor for creating quality-controlled products.

> *"The high dependency of CNC/CNF properties on their origin and the processing technique is a concern which needs to be considered regarding nanocellulose hydrogel preparation."* (Shojaeiarani et al., 2019)

Multifunctionality: As the scope of nanocellulose surface functionality increases, the potential for multifunctional hydrogel systems will increase accordingly. Stimuli-responsive hydrogels are already heralding this shift in functionality from passive to active drug delivery, with the potential for additional functions such as self-healing abilities, multidrug release, and biosensor capabilities in the future.

Safety: Safety has been comprehensively studied for nanocellulose and determined to be largely nontoxic in of itself, as seen in Table 9.1. However, novel nanocellulose surface functionalization may introduce moieties capable of inducing undesirable biological effects.

> *"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."* (Sheikhi et al., 2019)

Commercialization: Across all areas of nanocellulose research, scientific and industrial groups are working hard to scale up production and uncover new application areas for nanocellulose-based materials to meet the growing demands of this industry. The aforementioned challenges of reproducibility, quality control, and safety, as well as biomass procurement, technoeconomic viability, and social acceptance are relevant here. Nanocellulose must present superior properties, both in terms of material performance and sustainability, to displace conventional biomedical materials on the market.

> *The recently implemented large scale commercial production of CNCs and CNFs, standardization, purification and characterization protocols, increasingly positive outlook on the toxicity profile, and the establishment of greener, more industrially feasible, and more reproducible nanocellulose surface modifications and processing methods all suggest that the use of CNCs and CNFs in applications ranging from small scale medical-grade products through to larger-scale sorbent products is feasible* (De France et al., 2017).

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