

Cellulose Nanocrystals: Surface Modification, Applications and Opportunities at Interfaces

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Abstract: Cellulose nanocrystals (CNCs) are rod-like nano-scale particles that are widely available in nature and have recently gained great interest in both research and industry, due to their high strength, high crystallinity, high surface area, low density, biodegradability and low toxicity. CNCs can be easily extracted from natural cellulose sources and are broadly useful, for example in polymer reinforcement, paper manufacturing, and rheology modification. The high density of functional groups on the surface of CNCs allows various chemical surface modifications, which permit tuning the properties of CNCs over a wide range. This review gives a brief overview of surface chemical modification of CNCs, focusing especially on those often utilized for our own research, which focuses on some of the most prominent areas of interests of CNCs, notably polymer reinforcement, healable polymers, stimuli-responsive nanohybrids, Pickering emulsion stabilizers, viral inhibitors, and cholesteric liquid crystal assemblies.

Keywords: Cellulose nanocrystals · Colloidal liquid crystals · Nanocomposites · Nanotoxicology · Pickering emulsions · Surface modification · Viral inhibitors

1. Introduction

Nanomaterials have dominated materials science research over the last couple of decades and are playing an ever increasing role in our daily lives.^[1] As a result of the extremely high surface area and structural features with dimensions on the order of the wavelength of light, such nanoscale materials often exhibit unique optical, mechanical, and/or electronic properties, which are not accessible in micro- and macroscale analogues. Accompanying the developments in inorganic nanomaterials, both academic and industrial interest in the utilization of renewable raw materials, such as cellulose, for similar purposes has rapidly grown in the last 10 years.^[2] Cellulose, a linear biopolymer consisting of β -1,4-linked β -D-anhydroglucopyranose units, is the most abundant organic substance on Earth and a practically inexhaustible resource for the (future) production of biobased materials.

Likewise, in an increasingly digital world, there is a steadily decreasing need for paper, and this trend has fueled many innovations within the forest products industry, especially in the pulp and paper sector. Among those, cellulose-based nanomaterials, or nanocelluloses, have emerged as a new class of renewable building blocks for advanced functional materials.^[2c,3] These include both high-aspect-ratio cellulose micro- or nanofibrils (CMFs/CNFs) and rod-shaped cellulose nanocrystals (CNCs).^[4] The latter, in particular, display characteristic properties of anisotropic nanomaterials such as high elastic modulus, however with the added features of low density and nanoscale chirality.^[5] The lack of colloidal building block symmetry likely assists in the formation of cholesteric, or chiral nematic, liquid crystal (LC) phases. Such chiral phases are preserved in the solid state upon evaporation-induced self-assembly, making them one of the only nanomaterials available that can be used as templates to cast long-range chiral order within inorganic solids.^[3a] This opens the door to a number of potential applications including, but not limited to, novel substrates for asymmetric catalysis, enantioselective sorption media, nanomachines and chiral metamaterials.^[6]

Cellulose nanocrystals are most commonly prepared by strong acid-catalyzed hydrolysis, often involving sulfuric acid, which under the appropriate conditions selectively hydrolyzes the amorphous regions of macroscopic cellulose fibers, while leaving the crystalline regions in-

tact.^[2a] Typical dimensions of CNCs range from 50 nm to more than 1 μ m in length and 5–50 nm in width, depending on the source of cellulose fiber. Fig. 1 shows an atomic force micrograph (AFM) of CNCs produced by sulfuric acid hydrolysis of cotton fiber.^[7] CNCs are produced at both pilot and commercial scale in a few countries, such as the USA and Canada, through extraction from bleached chemical wood pulp *via* sulfuric acid hydrolysis.^[8] Hydrolysis with sulfuric acid provides the added benefit of excellent colloidal stability in water, as a result of the introduction of anionic sulfate half-ester groups on the CNC's surfaces, which also plays a role in stabilizing their cholesteric LC phases. Besides sulfate half-ester groups, CNC surfaces present numerous hydroxyl functional groups, which allow a wide range of chemical reaction pathways toward hybrid nanomaterials with useful properties, ranging from stimuli-responsiveness to antimicrobial capabilities. In addition, chemical surface modifications provide an effective means to transform the relatively hydrophilic CNCs into hydrophobic particles, thereby enabling their use in hydrophobic dispersants.^[9] The degree to which the hydroxyl groups of CNCs can be modified with other functionalities is commonly referred to as the degree of substitution (DS), in which the theoretical maximum DS is 3.0 per anhydroglucose monomer unit (AGU). One of the primary challenges in working with CNCs is their hydrophilic nature and the tendency to irreversibly aggregate upon drying, on account of hydrogen bonding,

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such that extensive preparation steps are required to achieve success in surface reactions that are hindered by the presence of water. This is usually accomplished either by 1) lyophilization, followed by re-dispersion, ultrasonication and filtration or 2) centrifugation-assisted solvent exchange. As an alternative, water-tolerant reaction pathways have also been developed in order to alleviate the need for such laborious preparation steps, which may also be difficult to scale up. In this short review, a synopsis of our work in surface chemical modification of CNCs for various applications is presented, along with some of the exciting research challenges and opportunities for CNCs at interfaces, which will be the focus of our work over the coming years.

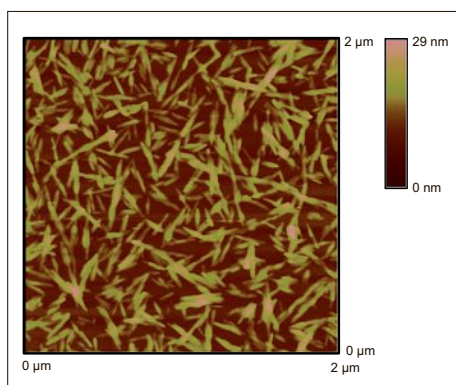


Fig. 1. AFM image ($2 \times 2 \mu\text{m}$) of CNCs produced from cotton fibers.^[7] Reproduced with permission of Springer.

2. Surface Chemical Modification of Cellulose Nanocrystals

There are a number of strategies to modify the surface of CNCs, depending on the target application, including oxidation, phosphorylation, silylation, amidation, (thio)carbamation, etherification, esterification, and polymer grafting.^[9,10] Given the high surface energy of CNCs, which is a result of the hydroxyl and sulfate half-ester groups, their surface chemical composition can vary between batches and different raw cellulosic materials, which may necessitate some pretreatment steps. Impurities that may be adsorbed on CNC surfaces include, for instance, proteins as well as side products from the acid-catalyzed hydrolysis process, such as aliphatic and aromatic compounds. Labet and Thielemans suggested that Soxhlet extraction of CNCs with ethanol prior to functionalization provided more reproducible results between different batches.^[11] Other pretreatment steps, such as hydrolytic desulfation, is another effective method to purify CNC surfaces and remove a portion of the anionic sulfate half-ester groups,

if they are indeed undesirable for certain reactions.^[12] In the following sections, an overview of the various chemical reaction pathways to subsequently modify CNCs in either aqueous or organic media will be introduced, while highlighting their resulting surface chemical properties and characterization methods.

2.1 Reactions in Aqueous Media

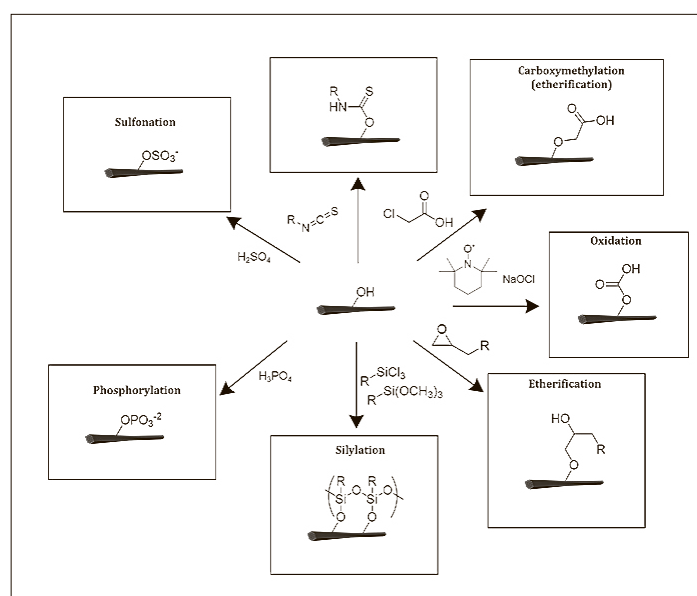
Of the numerous surface modification methods cited above, a few are tolerant, if not able to be carried out completely, in water. Scheme 1 provides an overview of various reaction pathways that can be used to modify CNCs in aqueous media. Given the heterogeneous nature of surface chemical modification of CNCs, the concentration of reactants required to obtain quantitative yields is usually several orders of magnitude higher than that of analogous reactions in homogeneous media.

One of the most widely investigated applications of CNCs is the use as mechanically reinforcing filler in synthetic and natural polymer matrices, *i.e.* the formation of nanocomposites.^[2d] CNCs hydrolyzed by sulfuric acid (S-CNCs) typically show a yellowish color when processed in nanocomposites at temperatures about 150°C or higher, or even at temperatures above 60°C when pure CNC films are heated. Though significant reinforcements of mechanical properties in nanocomposites processed at up to 170°C can be observed, coloration of normally colorless polymers, such as polyethylene, may not be desirable for specific applications. Moreover, polymers processed at even higher temperatures are not accessible for reinforcement with S-CNCs. In order to render CNCs more thermally stable, Espinosa *et al.* hydrolyzed CNCs using phosphoric acid (P-CNCs). This process affords phosphate

CNCs, which showed no yellowing below 180°C when heated in air (Fig. 2).^[10a] Neutral CNCs, or H-CNCs (obtained by hydrolysis with hydrochloric acid), show a similar degradation temperature, however they lack charged surface groups, which promote dispersibility due to electrostatic repulsion. P-CNCs combine the good dispersibility of S-CNCs and the thermal stability of H-CNCs and are thus an excellent candidate for incorporation into polymer matrices that are processed at high temperatures.^[13] Nevertheless, the surface charge density for P-CNCs of approximately 11 mmol/kg of cellulose is low compared to S-CNCs (60 mmol/kg of cellulose). Therefore, the long-term colloidal stability of S-CNCs in aqueous dispersions is expected to exceed that of P-CNCs.

An alternative strategy to generate CNCs that are stabilized by anionic surface functional groups is oxidation mediated by (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO).^[14] Way *et al.* utilized this method to functionalize CNCs with carboxylic acid groups to a surface charge density of 1080 mmol/kg .^[10b] The CNC-COOH thus obtained showed a pH-dependent dispersibility in water, as a result of electrostatic repulsion of CNC-COO⁻ under basic conditions, whereas hydrogen bonding induced aggregation and gel formation under acidic and neutral conditions (Fig. 3).

Another approach that we have used to functionalize CNCs is thiocarbamation of surface hydroxyl groups through reactions with isothiocyanates in 1 M NaOH for 20 h.^[15] Although this reaction requires alkaline conditions, *e.g.* pH 14, to achieve a detectable degree of substitution, it remains an effective means to graft small polar molecules to CNCs without the need for lyophilization or solvent-exchange. The success of this reaction was determined



Scheme 1. Overview of reaction pathways to modify CNCs that can be carried out in aqueous media.^[9c] Reprinted from ref. [9c]. Copyright 2017, with permission from Elsevier.

Fig. 2. Pictures of solution-cast samples of sulfated CNCs (S-CNCs, left), phosphated CNCs (P-CNCs, middle), and CNCs hydrolyzed by hydrochloric acid (H-CNCs, right) at different temperatures.^[10a] Reprinted with permission from ref. [10a]. Copyright 2013, American Chemical Society.

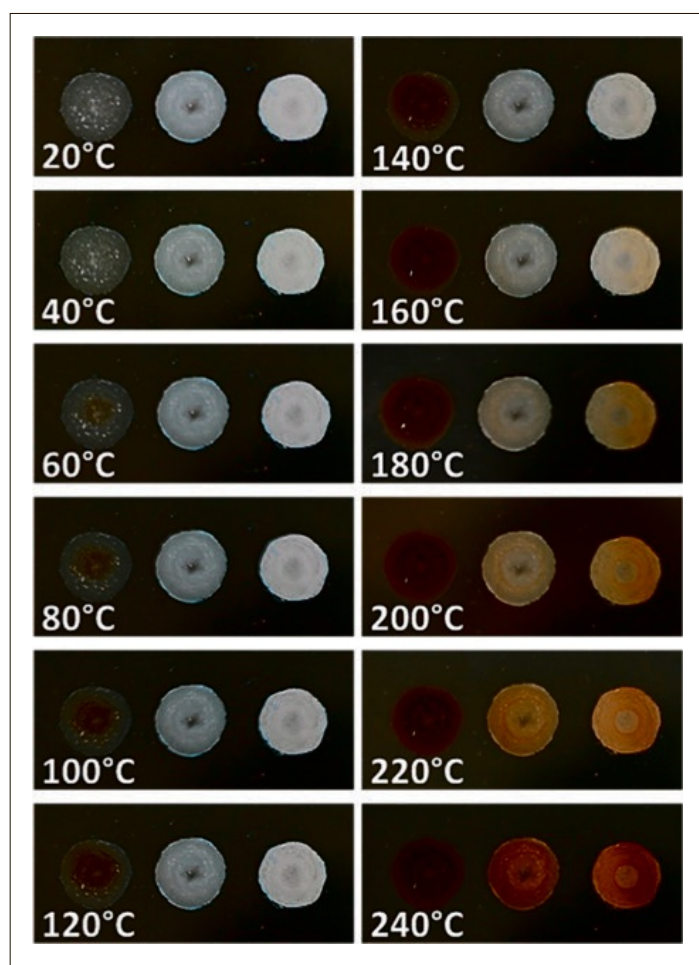
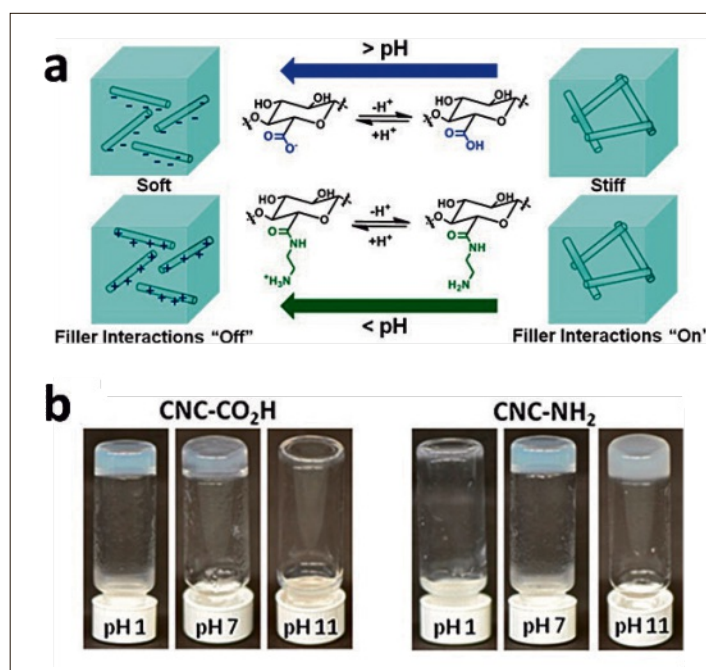


Fig. 3. (a) CNCs functionalized with carboxylic acid or amine groups show a decreased stiffness when in ionic form due to repulsing interactions at high pH (CNC-CO₂H) or low pH (CNC-NH₂). (b) Images of aqueous dispersions of CNCs functionalized with carboxylic acid (left) and amine (right) at different pH values.^[10b] Reprinted with permission from ref. [10b]. Copyright 2012, American Chemical Society.



by X-ray photoelectron (XPS) and FTIR spectroscopies. Similar conditions have also been used to convert CNC hydroxyl groups into epoxides through etherification with epichlorohydrin.^[16] Such epoxide-functionalized CNCs were further grafted with small homobifunctional amine cross-

linkers and, consequently, biomimetic ligands containing amine-reactive isothiocyanate and epoxide moieties. In addition to FTIR and XPS, the aromatic functional groups were also detected by UV/VIS spectroscopy. Our past efforts were primarily focused on grafting polymers

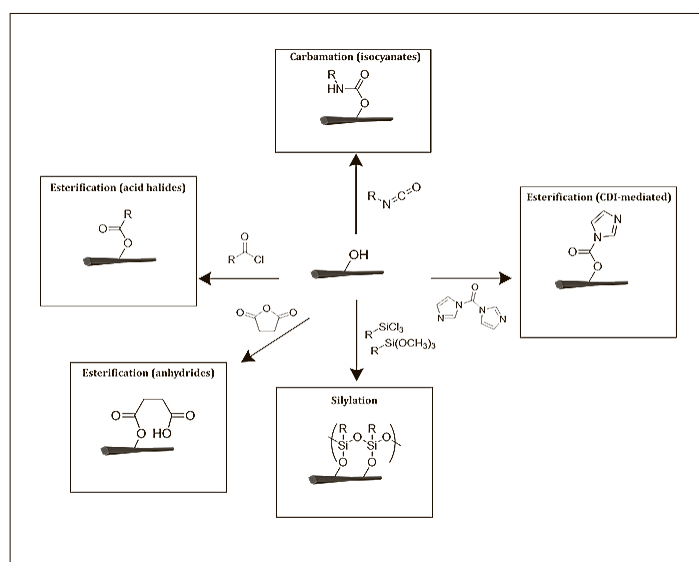
from CNCs *via* surface-initiated transition metal-mediated controlled radical polymerization, which requires the prior immobilization of appropriate initiators, which are often alkyl halides.^[17] Traditionally, this has been accomplished by esterification of surface hydroxyl groups with acyl halides in dry organic solvents.^[18] Inspired by the recent work of Boujemaoui *et al.*,^[19] we are currently developing water-tolerant protocols for initiator immobilization on CNCs based on acid-catalyzed Fischer esterification. An alternative water-tolerant route for polymer grafting also being explored in our lab is carbodiimide coupling of initiators containing *N*-hydroxysuccinimide (NHS) active esters to aminated CNCs.^[17a] We expect that the development of such water-tolerant protocols for grafting polymers from CNCs using controlled techniques will further facilitate both basic and applied research in the utilization of CNCs for advanced functional materials.

2.2 Reactions in Organic Media

Although a relatively wide range of water-tolerant reaction pathways exist to functionalize the surface of CNCs, the chemist's toolbox can be greatly expanded when such reactions are conducted in organic media. Scheme 2 provides an overview of common reaction pathways in organic dispersants. As mentioned earlier, either lyophilization or centrifugation-assisted solvent exchange is usually a prerequisite to disperse CNCs in an organic solvent, if moisture-sensitive reactions are to be carried out.

We have focused a great deal on functionalizing CNCs based on isocyanate chemistry, due to the highly reactive nature of isocyanate functional groups toward hydroxyls. Schyrr *et al.* used isocyanate chemistry to derivatize CNCs with biosensing moieties.^[20] Porous thin films of polyvinylalcohol and CNCs were cast on a glass surface and a subsequent two-step functionalization with different sensing molecules was performed, using toluene-2,4-diisocyanate (2,4-TDI) as a linker. CNCs were functionalized with the aromatic isocyanate molecule containing an unsaturated double bond as an end group, which permitted subsequent thiol-ene Michael addition of the sensing moiety. Mild reaction conditions (45 °C, DMF, stirring overnight) were used, yet a high DS of 0.12 was achieved, which corresponds to a functionalization of approximately 65% of all primary available hydroxyl groups on the CNCs' surface. A similar approach involving 2,4-TDI was utilized to graft CNCs solvent-exchanged to dry toluene in the presence of trimethylamine (75 °C, 24 h) and render CNCs reactive toward polymers with terminal hydroxyl groups.^[21]

Scheme 2. Overview of common reaction pathways to modify CNCs that are carried out in organic media. [9c] Reprinted from ref. [9c]. Copyright 2017, with permission from Elsevier.



Isocyanate-based modification of CNCs was also used by Biyani *et al.*, who functionalized CNCs with coumarin in order to produce photoswitchable nanocomposites.^[22] Reacting a hydroxyl-functionalized coumarin derivative with an excess of hexamethylenediisocyanate (HMDI) resulted in the precursor (Cou-NCO), which was subsequently reacted with CNCs for 24 h in DMF at 100 °C using dibutyltin dilaurate (DBTDL) as a catalyst. Several centrifugation cycles were carried out in order to remove unreacted Cou-NCO and FTIR spectroscopy was used to qualitatively confirm the success of the reaction, which was seen in the appearance of a characteristic peak associated with the carbonyl moiety at 1700 cm^{-1} . Also a mass increase of the CNCs indicated a successful modification and UV/VIS spectroscopy was used to quantitatively determine the degree of substitution. By comparing the absorption of Cou-CNC at 318 nm with the absorptions of solutions with known concentrations of Cou-NCO, a coumarin content of 217 mmol kg^{-1} was calculated for the Cou-CNCs. Biyani *et al.* also explored light-healable supramolecular nanocomposites based on the reversible quadruple hydrogen bonding of ureidopyrimidinone (UPy) motifs.^[23] Some organic solvents such as DMF prevent UPy motifs from dimerizing, and thus the surface modification was performed in DMF at similar conditions as described above to prevent dimerization and CNC agglomeration during the modification step. Following the same procedure, Natterodt *et al.* attached UPy motifs to CNCs and explored the adaptable behavior of CNC-UPy by incorporation into both polar and non-polar polymer matrices.^[24] While the good dispersibility of the polar CNC-UPy motifs in polar solvents was as expected, CNC-UPy interestingly also showed a good dispersibility in non-polar solvents such as toluene, allowing solution

casting with commercial polymers such as polyethylene (Fig. 4). In addition to previously mentioned characterization methods for the surface modification, the increase of nitrogen content was monitored by elemental analysis, which resulted in a degree of substitution of 0.17 in good consistency with the value obtained by UV/VIS titration (0.16).

The functionalization of CNCs with a mechanochromic linker that can form radicals upon mechanical activation as demonstrated by a color change from light to dark blue was reported by Imato *et al.*^[25] The diarylbibenzofuranone (DABBF)-modified CNCs are in an equilibrium with their dissociated, radical form, which can be shifted to a higher percentage of radicals by mechanical activation (grinding) resulting in an enhanced coloration. Compared to a similar system without CNCs, the mechanochrome showed an increased radical stability when attached to the CNCs, probably due to the limited molecular mobility. Because of the temperature sensitivity of DABBF, the reaction between CNCs and

a diisocyanate DABBF derivate was performed at only 40 °C for 72 h, and the degree of substitution was moderate (0.015).

With the goal to create pH-responsive gels and nanocomposites, Way *et al.* produced amine-functionalized CNCs in a three-step process.^[10b] The first step was the derivatization of CNCs with carboxylic acids using TEMPO-mediated oxidation as described above, followed by the esterification with a mono-protected diamine and subsequent deprotection of the primary amine. The esterification was performed using a standard protocol by using an excess of the amine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 4-dimethylaminopyridine (DMAP). Conductometric titration of the remaining carboxylic acids was used to determine the reaction yield of 71%. Activation of CNC hydroxyl groups with 1,1'-carbonyldiimidazole (CDI) has also been utilized in our lab in order to render CNCs amine reactive.^[15] Such reactions were conducted in dry DMSO at 40 °C for 20 h. Homobifunctional amine crosslinkers were then reacted at 40 °C for another 20 h in order to ultimately graft isothiocyanate-functional biomimetic ligands to CNCs for inhibition of alphaviruses to an ultimately higher DS than what was achieved by reacting isothiocyanates directly with CNC hydroxyl groups in aqueous media.

Molecules containing acyl chloride moieties for the attachment and following release of pro-fragrances from CNCs were synthesized by Kuhnt *et al.*^[26] This approach allowed the base-induced slow release of the fragrances *via* a retro 1,3-Michael-type reaction as it might be useful in long-lasting perfumes. The modification *via* acyl chlorides resulted in a weight increase of only *ca.* 1.6% w/w, which is much lower than what is typically obtained with isocyanate chemistry, however the fragrance would have likely been cleaved from the CNCs under such conditions.

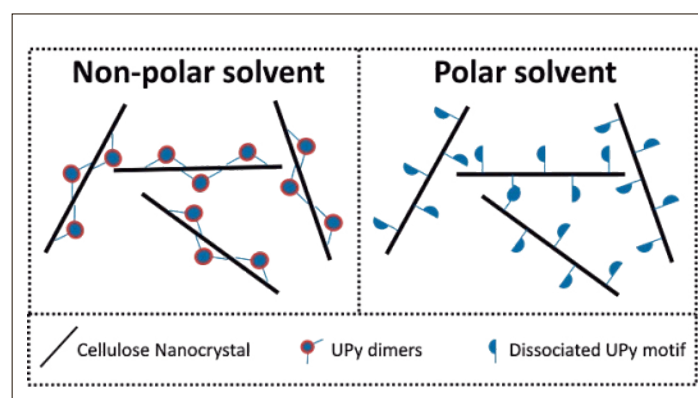


Fig. 4. The good dispersibility of CNC-UPy in both non-polar and polar solvents is as a result of a decreased polarity of CNC-UPy in non-polar solvents forming intra-CNC dimers (left) and a high polarity due to free UPy motifs in polar solvents (right).^[24] Reprinted with permission from ref. [24]. Copyright 2017, American Chemical Society.

2.3 Polymer Grafting

Polymer grafting is a highly effective means to control the interfacial properties of solid surfaces, especially for applications in which the long-term stability of thin polymer films is crucial, such as biomedical devices and stimuli-responsive surfaces.^[17] There are two main pathways to graft polymers on solid interfaces, namely ‘grafting to’ and ‘grafting from’ methods. In the ‘grafting to’ approach, a pre-synthesized polymer containing a reactive end functional group is covalently linked to a functional group presented at a solid interface. Grafting a full-length polymer chain to a substrate may experience steric hindrance at a certain interchain distance that ultimately limits the thickness and grafting density of the coating. On the other hand, the ‘grafting from’ method is a two-step process involving first the covalent attachment of a small molecular initiator to the surface, followed by the introduction of monomers/catalyst in order to synthesize polymer chains directly from the solid substrate. This approach has the advantages of high polymer grafting density and easily controlled film thickness. These so-called ‘polymer brushes’ produced in the ‘grafting from’ manner also provide unique properties as a result of their highly stretched chain conformation.

We have used the ‘grafting to’ approach in order to compatibilize CNCs with hydrophobic poly(ϵ -caprolactone) (PCL) nanofiber matrices.^[21] This reaction pathway required the initial activation of CNC hydroxyl groups with 2,4-TDI in order to covalently attach a low molecular weight PCL diol, shown in Scheme 3. This two-step process was conducted in dry toluene at 75 °C and in the presence of TEA over a total of 6 days, due to the unfavorable steric hindrance encountered when grafting the 2 kDa PCL diol to CNCs. With a grafting yield of 30%, the PCL diol-modified CNCs were indeed rendered hydrophobic and readily dispersed in dichloromethane.

Due to the relatively low surface grafting density that can be obtained using ‘grafting to’ methods, we have directed our research efforts towards ‘grafting from’ the surface of CNCs through surface-initiated controlled radical polymerization (SI-CRP).^[17a] Of the various controlled polymerization techniques, those mediated by transition metal complexes, such as copper, are the most frequently employed due to the relatively simple experimental setup and tolerance toward a wide variety of functional groups. Copper-mediated polymerizations, such as atom transfer radical polymerization (ATRP), requires the prior covalent immobilization of alkyl halide initiator on CNC surfaces. At first, we used a centrifugation-assisted solvent exchange procedure (from water to dry DMF or

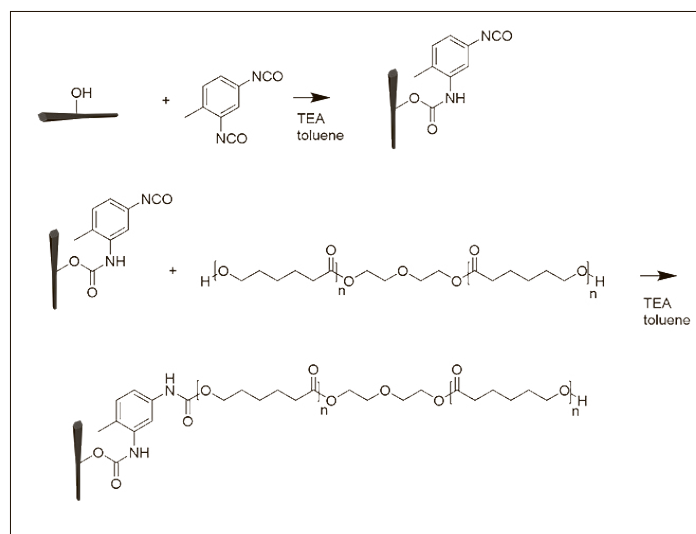
THF) in order to graft α -bromoisobutyryl bromide (BiBB) to CNC surface hydroxyl groups *via* 4-DMAP catalyzed esterification (Scheme 4).^[12,27] These initiator-modified CNCs were consequently utilized to grow poly(*N*-isopropylacrylamide) (PNIPAM)^[27] and poly(*N,N*-dimethylacrylamide) (PDMAM) chains to a number-average molecular weight (M_n) of up to 43 kDa and 530 kDa, respectively.^[27] The molecular weight of surface-tethered polymers was determined by cleaving them from the CNC substrates by alkaline hydrolysis of ester bonds and carrying out size exclusion chromatography (SEC) analysis. In our investigation on the effect of anionic sulfate half-ester groups on SI-ATRP of DMAM, we found that surface charge, although providing higher surface-bound initiator efficiency, had a negative impact on the dispersity of the resulting polymer grafts, *i.e.* leading to higher M_w/M_n . This was explained by the partial dissociation of copper complexes in aqueous media that become immobilized by sulfate groups and thus cannot efficiently deactivate propagating chains.^[12]

As mentioned in section 2.1, we are currently focusing on water-tolerant protocols for initiator immobilization on CNCs based on acid-catalyzed Fischer esterification^[19] and carbodiimide coupling of initiators containing *N*-hydroxysuccinimide (NHS) active esters to aminated CNCs in order to expand the scope of copper-mediated SI-CRP from CNCs toward more robust liquid crystal phases.^[17a]

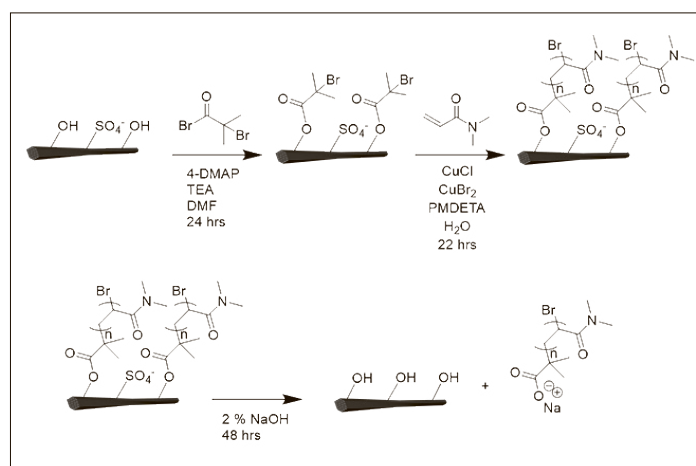
3. Applications

3.1 Nanocomposite Materials

The chemical surface modification of CNCs enables exploiting their unique properties in a wide range of applications, such as nanocomposite materials, thin films, hydrogels, Pickering emulsions, and biomedical devices.^[2b,9] Surface-modified CNCs find diverse applications in polymer nanocomposites, not only on account of their potential to improve the mechanical properties, but also because the properties of CNCs can be tuned by attaching molecules with different functions.^[9] Nicharat



Scheme 3. Grafting of PCL diol to CNCs *via* 2,4-TDI activation of hydroxyl groups.^[21]



Scheme 4. Surface-initiated atom transfer radical polymerization (SI-ATRP) of *N,N*-dimethylacrylamide (DMAM) from CNCs and subsequent cleavage of PDMAM chains from CNC surfaces *via* alkaline hydrolysis.^[12] Reprinted with permission from ref. [12]. Copyright 2016, American Chemical Society.

et al. prepared nanocomposites of polyamide 12 with P-CNCs *via* melt-processing based on the excellent thermal stability of phosphorylated CNCs.^[28] With a processing temperature of 190 °C, the same polymer matrix showed strong coloration when mixed with S-CNCs and almost no coloration when mixed with P-CNCs, and additionally showed slightly increased mechanical properties with P-CNCs compared to S-CNCs. The lyotropic liquid crystal order adopted by CNCs in concentrated dispersions and free-standing films has also been exploited in nanocomposites to produce so-called liquid crystalline thermosets (LCTs).^[7] An attractive feature of LCTs is that they display enhanced mechanical properties, as a result of heterogeneities and localized anisotropy within an overall isotropic microstructure. This morphology has been suggested to cause non-linear crack propagation and thus increased modulus and toughness. We have employed this approach to water-miscible epoxy precursors, namely 1,4-butanediol diglycidyl ether (BDGE) and 2,2'-(ethylenedioxy)bis(ethylamine) (EBEA), which were mixed with concentrated CNC dispersions, followed by slow solvent evaporation and curing, in which the CNC hydroxyl groups can also react with the epoxide moieties. This process led to nanocomposites with 50–72% w/w CNCs embedded in an elastomeric matrix with *ca.* 1 GPa elastic modulus and T_g of –25 °C. Not only did the CNC-based LCTs exhibit mechanical properties similar to traditional LCTs, but also various degrees of optical birefringence as a result of anisotropic CNC phases.

The derivatization of CNCs with hydrogen-bonding UPy groups has afforded a ‘universal’ filler that can readily be compounded with diverse (polar and apolar) polymer matrices and afforded a range of nanocomposites with enhanced mechanical properties.^[6] Another example is an approach towards self-healing polymers, which are generally very soft, by increasing their mechanical properties through the addition of functionalized CNCs, while maintaining their self-healing ability.^[23,29] We have also reported a number of stimuli-induced changes of diverse nanocomposite properties (mechanical, optical, healing, *etc.*). For instance, the functionalization of CNCs with a photoactive motif, coumarin, followed by incorporation into a polymer results in increased mechanical properties compared to the neat polymer, which can be even further increased by irradiation if the photoactive motif is chosen to associate upon radiation.^[22] Upon irradiation with UV light, the coumarin moieties on the surface of two different CNCs dimerized and thus resulted in enhanced inter-CNC interactions and increased mechanical

properties. The storage modulus was increased from 4 MPa for the neat polymer (ethylene oxide–epichlorohydrin copolymer) to 199 MPa with 10% w/w functionalized non-irradiated CNCs to 291 MPa after UV-radiation. On the other hand, when the surface groups were designed to dissociate upon heating, as in the case of the hydrogen-bonding self-complementary UPy-motif, thermal or UV-light induced healing can be achieved.^[23] For example, the UPy-modified CNCs were incorporated into a supramolecular polymer matrix composed of UPy-terminated poly(ethylene-*co*-butylene) telechelics, and the mechanical properties of the nanocomposite thus obtained were significantly increased compared to the neat polymer. Irradiation with UV-light caused instant heating of the material (through a light–heat conversion process), which in turn caused the dissociation of the UPy motif and led to liquefaction of the material. This process is completely reversible and the material solidified upon cooling. This process permits healing small scratches in the material quickly, efficiently, and in a spatially resolved manner. Another interesting approach is the design of a motif which will remain inert under acidic conditions (for example during storage), but respond to higher pH environments useful for controlled release of a fragrance.^[26] Even though many of these stimuli-induced property changes can also occur without the presence of CNCs, CNCs can serve as a nanocarrier and thus allow the use of the designed motif in diverse systems such as polymer nanocomposites, on surfaces or in solutions. Stimuli changing nanocomposites are not limited to light, temperature or water, but can include a much broader area such as bioorganisms which lead to an increase in fluorescence of CNCs functionalized with bioorganisms sensing motifs.^[20] Depending on the attached molecules, fluorescence dependence on the pH could be monitored, or the addition of a protease could cleave a fluorescence-quenching peptide and thus increase the fluorescence intensity as a function of time.

The use of CNCs in nanocomposites also extends from bulk polymers and thin films to polymer nanofibers produced by electrospinning.^[21,30] Some of the applications of nanofiber mats, such as tissue engineering scaffolds, often benefit from the use of nanofillers in order to increase their mechanical strength. One such polymer matrix that is widely used for biomedical applications due to its biodegradability and biocompatibility is poly(ϵ -caprolactone) (PCL). However, nanofiber mats of PCL often have low mechanical strength, which can limit their use in engineering tissues that are subjected to frequent mechanical impact. Our group has utilized both

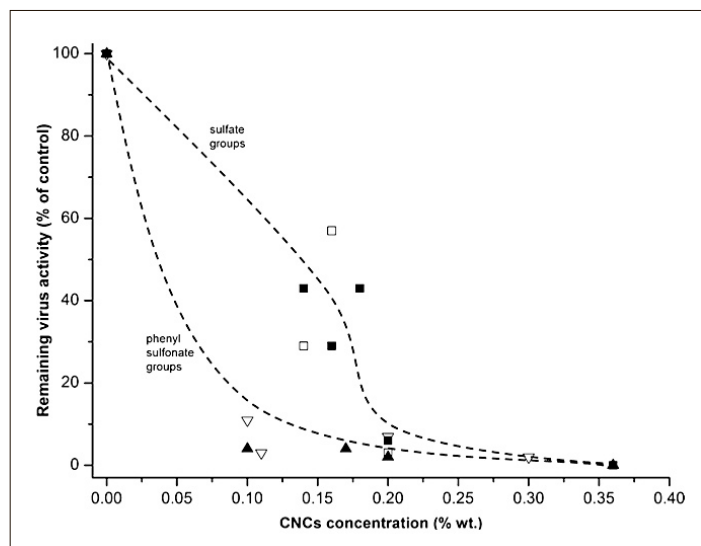
unmodified and modified CNCs as reinforcement in PCL nanofibers.^[21] While the use of unmodified CNCs resulted in PCL nanofiber composites that displayed a *ca.* 1.5 fold increase in modulus compared to neat nanofibers and which maintained their structural integrity, CNCs modified with a low-molecular-weight PCL diol disrupted the crystallization of the PCL nanofibers during electrospinning, resulting in heterogeneous morphology and impaired mechanical properties. In this case, the surface of unmodified CNCs more effectively nucleated the crystallization of the PCL matrix.

3.2 Viral Inhibitors and Nanotoxicology of Cellulose Nanocrystals

The antimicrobial properties of polyanionic compounds, including sulfated polysaccharides, is well-established and they have been thoroughly considered as active ingredients in topical microbiocides for the prevention of various infections, including human immunodeficiency virus-1 (HIV-1).^[15,16] The facilitation of HIV entry into host T-cells and macrophages is known to take place *via* binding of sulfated tyrosine residues at the *N*-terminus of chemokine receptor 5 (CCR5) to the envelope glycoprotein 120 (gp120). These precedents inspired our group to develop so-called ‘tyrosine sulfate mimetic’ nanostructures based on CNCs for application as viral inhibitors. The inherent sulfate half-ester groups presented on CNCs derived from sulfuric acid hydrolysis may indeed mimic tyrosine sulfate interactions with enveloped viruses and functionalization with phenyl sulfonate groups may provide more specificity toward envelope binding domains. In preliminary studies with model alphaviruses genetically engineered to express enhanced green fluorescent protein, namely Semliki Forest virus vector (VA7-EGFP), both unmodified CNCs containing only the inherent sulfate half-esters and CNCs carrying multivalent displays of phenyl sulfonate groups were highly effective inhibitors *in vitro* (Fig. 5).^[15] We observed that CNCs with phenyl sulfonate groups were roughly twice as effective as CNCs featuring sulfate half-ester groups, in which only 0.1% w/w CNCs with phenyl sulfonates inhibited nearly 100% of the viral vector. We hope to expand future work on such antiviral properties toward other enveloped viruses, *e.g.* HIV-1, and develop new assays based on surface-sensitive analytical techniques in order to rapidly screen potential CNC-based inhibitors *via* surface plasmon resonance (SPR) and quartz crystal microbalance with dissipation monitoring (QCM-D).

The potential biological impact of CNCs, in both wet and dry conditions,

Fig. 5. Influence of cotton CNCs bearing sulfate groups or phenyl sulfonate groups as a function of concentration on the inhibition of VA7-EGFP infection *in vitro*. Legend: cotton CNCs batch 1 (■) and batch 2 (□), cotton-SPTC (▲) and cotton-EBEA (▽). Note: Dotted lines drawn to guide the eye.^[15] Reprinted with permission from ref. [15]. Copyright 2014, American Chemical Society.



is a crucial factor in assessing their suitability for applications in various areas of nanomedicine. In the above work, neither unmodified nor modified CNCs caused observable *in vitro* cytotoxicity to Vero (B) cells or human corneal epithelial cells (HCE-T) within the concentration range investigated, *i.e.* up to 0.4 % w/w.^[15] Previous toxicology studies of cotton-based CNCs indicated that their cytotoxicity is significantly lower than that of multiwalled carbon nanotubes and crocidolite asbestos fibers.^[31] These findings have been further supported by later assessments, although the specific characteristics of CNCs that vary between sources, such as crystallite dimensions, surface charge/functionality, as well as their concentration and interactions with different polymer matrices can largely influence the toxicity of CNCs.^[32] In order to assess the inhalatory risk of airborne CNCs, Endes *et al.* developed an Air Liquid Interface Cell Exposure system (ALICE).^[33] A more general approach was proposed by Camarero-Espinosa *et al.*, who suggested a set of guidelines to fully deduce the potential human risk of cellulose-based nanomaterials, in general, considering what is already known regarding the toxicity of other classes of nanomaterials.^[34] All the above considered, current laboratory use of CNCs must be performed under high safety standards. Nevertheless, they remain as promising candidates for applications that involve interfacing with biological systems.

3.3 Pickering Emulsion Stabilizers

The unusually high stability of emulsions prepared using solid particles, known as Pickering emulsions, in the place of conventional surfactants is a result of the large energy barrier that must be overcome to desorb particles from the liquid–liquid interface and induce droplet coalescence.^[35] The fact that the different crystal faces of

CNCs have a varying degree of hydrophobicity and highly tunable surface charge makes them excellent candidates for stabilizing oil–water interfaces.^[36] Surface chemical modification with stimuli-responsive polymers provides the additional benefit of an external trigger to break Pickering emulsions on demand, useful for controlled delivery of hydrophobic compounds.^[27,37] Such is the case for CNCs grafted with PNIPAM, which exhibit lower critical solution temperatures (LCST) in water dependent on grafting density, molecular weight and ionic strength of the medium. This class of CNC hybrid was used to provide stable (*ca.* 4 months) heptane-in-water Pickering emulsions.^[35] On the other hand, unmodified CNCs containing sulfate half-ester groups were unable to stabilize the emulsions. The collapse of PNIPAM grafts could be triggered in order to break emulsions on demand

upon heating above the LCST. Besides phase separation visible to the naked eye, this was also demonstrated by monitoring Pickering emulsion viscosity as a function of temperature, shown in Fig. 6, in which a temperature and concentration dependent change in viscosity was observed.

4. Opportunities of Cellulose Nanocrystals at Interfaces

Thus far, we have highlighted just a few of the applications of modified CNCs in which we have been involved. In our view, one of the most intriguing future directions in CNC research is better control over their cholesteric liquid crystal phases, which are emerging as templates for novel solid-state materials with long-range chiral order.^[3a] Liquid crystals (LCs), in general, are widely used in the opto-electronic industry for displays and other applications due to their unique combination of optical, rheological and electrical properties.^[38] Cholesteric (N*), also known as chiral nematic, LCs are those in which the nematic director rotates in the form of a helix, which can be right- or left-handed. A fundamentally interesting observation is that although CNCs individually exhibit right-handed twists,^[5] they always form left-handed N* phases. Right-handed N* phases have, thus far, never been observed. Moreover, the link between microscopic and macroscopic chirality in such anisotropic colloidal systems is still not fully understood,^[39] though is essential to developing colloidal building blocks for assembling complex 3-dimensional architectures.^[40] Among the external factors that can be used to manipulate N* phases of CNCs, such as ionic strength, ultrasound, *etc.*, surface chemi-

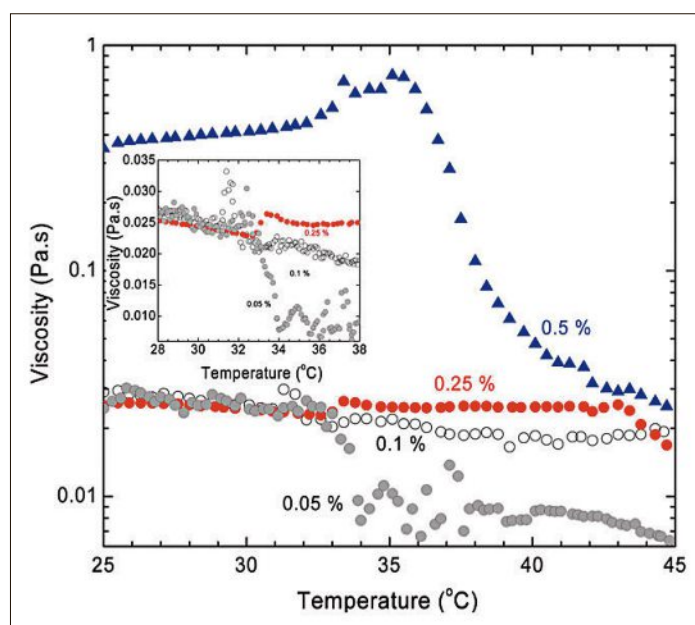


Fig. 6. Viscosity (100 s⁻¹) versus temperature for Pickering emulsions prepared with 0.05 (gray circles), 0.1 (open circles), 0.25 (filled circles), and 0.5 (triangles) % w/w CNCs-g-PNIPAM (note semi-log scale). Inset: enhanced view of 0.05, 0.1 and 0.25 % w/w CNCs-g-PNIPAM (linear scale).^[35] Reprinted from ref. [35]. Copyright 2012, with permission from Elsevier.

cal modification of CNCs with tethered polymers remains less explored.^[2a] This is probably because non-selective surface modification may have a negative impact on the formation of N* phases, as the packing density of CNCs would be disturbed by the presence of macromolecular layers. On the other hand, selective modification at the ends of CNCs with polymer tethers would still allow sufficient packing within N* phases and may offer a means to control N* phase pitch, handedness and, in general, make CNC chiral assemblies more robust to different chemical environments. The unique features that make CNCs a promising platform for such nano-hybrids are the molecular directionality of individual cellulose chains and existence of reducing end groups presenting reactive aldehydes, the locations of which can be varied depending on the cellulose crystal polymorph.^[41] Multiple research groups have already demonstrated the feasibility to modify CNC reducing ends through various reaction pathways leading to CNCs with thiol end groups.^[42] Sipahi-Saglam *et al.* also already reported the free radical polymerization (FRP) of acrylamide from CNC reducing end groups,^[43] however, numerous opportunities still exist to build upon these works. The major thrusts of our research in the coming years will be the investigation of the morphology (chirality) of CNCs with different crystal structures, SI-CRP from initiator-functionalized reducing end groups and the effect of end-tethered polymer chains on the formation of N* LC phases in various solvents. Through the well-known chemical transformations of cellulose crystallites that alter the distribution of reducing end groups, we hope to develop both symmetric and asymmetric CNC hybrids with end-tethered polymer chains, similar to inorganic Janus particles.^[44] Given the recent emergence of chirality in nanoscience and nanotechnology,^[6b,c] we foresee CNC hybrids playing a crucial role in accessing new chiral materials through templating.^[3a] Far on the horizon, one could imagine that with appropriately chosen end-tethered polymers, metallic nanoparticles could be site-selectively immobilized on CNCs, and thus potentially providing new nanohybrid platforms for bottom-up assembly of chiral metamaterials.^[6a]

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