

Cellulose Nanofibrils: From Strong Materials to Bioactive Surfaces**

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ABSTRACT: Cellulose nanofibrils (CNF), also known as nanofibrillar cellulose (NFC), are an advanced biomaterial made mainly from renewable forest and agricultural resources that have demonstrated exceptional performance in composites. In addition, they have been utilized in barrier coatings, food, transparent flexible films and other applications. Research on CNF has advanced rapidly over the last decade and several of the fundamental questions about production and characterization of CNF have been addressed. An interesting shift in focus in the recent reported literature indicates increased efforts aimed at taking advantage of the unique properties of CNF. This includes its nanoscale dimensions, high surface area, unique morphology, low density and mechanical strength. In addition, CNF can be easily (chemically) modified and is readily available, renewable, and biodegradable. These facts are expected to materialize in a more widespread use of CNF. However, there is no clear indication of the most promising avenues for CNF deployment in commercial products. This review attempts to illustrate some exciting opportunities for CNF, specifically, in the development of aerogels, composites, bioactive materials and inorganic/organic hybrid materials.

KEYWORDS: Cellulose nanofibrils, CNF, nanofibrillar cellulose, NFC, cellulose materials, aerogels, hydrogels, composites, bioactive surfaces, inorganic/organic hybrids

1 INTRODUCTION

Cellulose, the most abundant biopolymer on earth, has been studied thoroughly for different applications including reinforcement of hydrophobic polymeric matrices, biomedical implant and composite materials, among others. Cellulose is composed of repeating units of cellobiose consisting of anhydroglucose linked together by β -1,4 linkages (Figure 1). Unlike starch and other polysaccharides, the β -1,4 glucosidic bond in cellulose causes the macromolecular chains to align straight and for the hydroxyl groups at given positions to hydrogen bond within (intra-chain hydrogen bonding) and between the polymer chains (inter-chain hydrogen bonding). Hydrogen bonding in cellulose affects solvency, swelling and key physical properties of cellulosic structures (Figure 1).

Cellulose nanofibril (CNF) is a material containing fibrils with length in the micrometer and width in the nanometric range, forming a network structure. CNF can be prepared by its liberation from the constituent fiber matrix and microfibril bundles [1–3]. The nanofibrils contain both amorphous and crystalline cellulosic regions [4]. This fibrillar material should be differentiated from crystalline cellulose, referred to as cellulose nanocrystals (CNC) or nanowhiskers (Figure 1). CNCs are prepared from fibers and fibrils via acid hydrolysis that degrades the amorphous regions, yielding highly crystalline nanoparticles [5].

CNF is prepared by mechanical methods via high shearing followed by homogenization at high pressure. The equipment commonly used to produce CNF includes high pressure homogenizers, refiners, grinders, cryo-crushers, and microfluidizer. A thorough description of these techniques has already been reported in a review by Lavoine *et al.* [6]. Reduction of energy consumption has been a primary focus in the production of CNF; thus, several methods have been developed including chemical, mechanical or enzymatic fiber pretreatment. A comprehensive description of these

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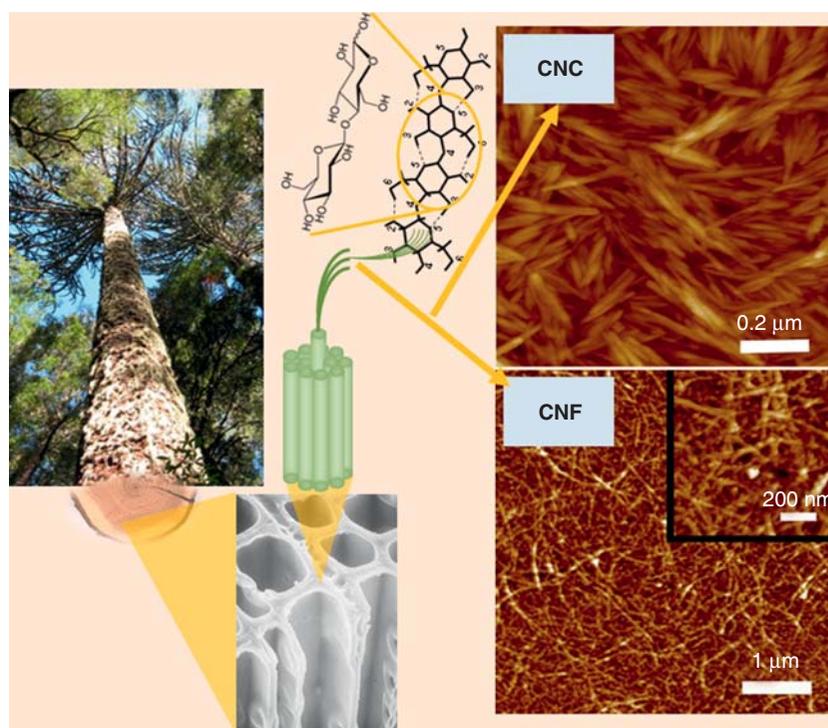


Figure 1 Schematic illustration of cellulose nanofibrils (CNF) obtained from wood fibers after deconstruction of the highly hierarchical, multidimensional structure in wood. The major molecular component of the respective building blocks in fibers is the cellulose polymer (note the repeating cellobiose unit of cellulose and the intra-chain bonds). The fibril structures made from cellulose can be disassembled to produce CNF, but if the amorphous regions of the fibrils are removed by acid hydrolysis, cellulose nanocrystals are obtained (CNC). Note that nanocellulose from other sources may occur in different packing as dictated by the (bio)synthesis in nonwood plants, microorganisms, algae and sea animals. Some reported sources in addition to bleached softwood and hardwood pulps include cotton, ramie, hemp, flax, sisal, wheat, straw, palm, sugar beet pulp, bacterial cellulose, *Valonia* algae, tunicates, etc.

pretreatments can be found elsewhere [2, 6–9]. Besides wood and plant-based CNF, the fibrils can be prepared by bottom-up approaches using biosynthesis by microorganisms [10]. Under specific culturing condition, bacterial cellulose (BC) is produced by the gram-negative bacteria *Acetobacter xylinum* (or *Gluconacetobacter xylinum*), as extracellular cellulose, where the fiber material is amenable to extraction [10].

Since the development of CNF from wood in the 1980s, along with the progress of cellulosic fibril research, the nomenclature has changed. When the material was first introduced it was referred to as microfibrillated cellulose (MFC) [1, 3]. Since then varying approaches have been utilized to prepare the fibrillar material with different degrees of deconstruction, leading to materials with characteristic sizes in the micro and nano scales. Typically MFC consists of fibers with fibrillated surfaces, whereas CNF is composed of fibrils or fibril bundles that are separated as nanosized entities. However, micron-sized fibrils are loosely referred to as CNF. In addition to CNF the nanomaterial can also be called nanofibrillar cellulose (NFC).

Applications taking advantage of physical, mechanical and chemical features of CNF are being explored in academia and industry. Some of the current applications of nanofibrillar cellulose are highlighted schematically in Figure 2. Due to their surface properties, CNF fibrils are able to bind significant amounts of water inside the fibril network, forming a hydrogel. This phenomenon is beneficial in applications as a rheology modifier [3] or in wound dressing [11]. However, the high water content of the material makes preparation of structures such as solid films and coatings a challenge. Despite this limitation, CNF has been extensively explored in the preparation of transparent films [12–14]. In addition to optical applications, CNF offers potential in packaging efforts to replace oil-based materials due to the fact that the crystallinity and the network structure of CNF make it attractive in applications demanding barrier properties. CNF can be used as a strength agent [15–17], can be combined with polyelectrolytes in fiber processing [18–21] and can be used in the fabrication of composites [22–24]. Finally, CNF has great potential in

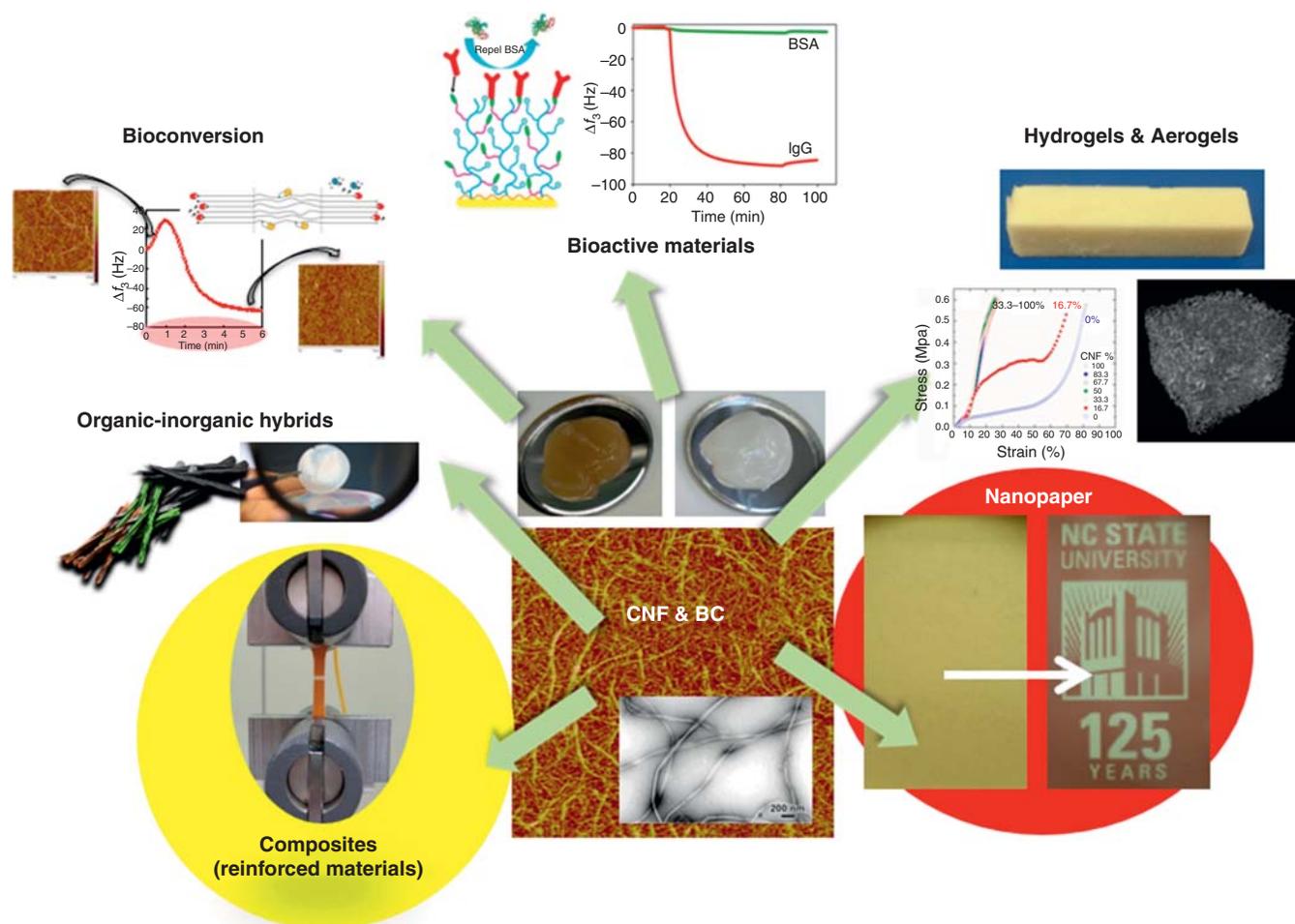


Figure 2 Current and potential applications of CNF and BC, including composites, organic-inorganic hybrids, gels, foams, aerogels/xerogels, coatings and nanopaper, bioactive and wound dressing materials, bioconversion, etc.

the biomedical field. A detailed account of the role of CNF in the applications reported in the literature is a challenging task. In this review a few aspects related to the use of CNF are discussed, namely, in the production of aerogels, composites, bioactive materials and inorganic/organic hybrids (see Figure 2).

2 CNF AEROGELS

Aerogels are ultra lightweight materials that are formed by the removal of liquid solvent from a gel without collapsing the network structure [25–27]. These types of materials were first introduced in 1931 [28] and are often referred to as solid foams (when air bubbles are introduced in the gel), frozen smoke (common name for silica aerogels), cryogels (aerogels produced by freeze-casting), porous nanopapers (porous thin materials obtained after drying cellulose nanofibril gels) and xerogels (obtained from air-drying). Aerogels can be inorganic, inorganic-organic and

organic. Most commonly they are prepared by sol-gel polymerization of inorganic metal oxides [29].

Interest in aerogels stems from their unique properties, which include low density ($0.004\text{--}0.5\text{ g cm}^3$), high porosity (above 80%), high specific surface area, low thermal conductivity and low dielectric permittivity [25–27]. Aerogel formation, usually in aqueous medium, can be induced by a chemical (reaction) or physical (pH, temperature) crosslinking. Drying the wet gels is critical for most of the aerogel properties; the most common procedures used include supercritical carbon dioxide drying [29], freeze-casting [30], and vacuum-drying [31]. Inorganic aerogels are usually very brittle; therefore hybrid aerogels have been developed with organic polymers in order to improve their elastic properties.

Cellulose is an attractive polymer for the production of bio-based aerogels due to its biodegradability, biocompatibility, availability, renewability and capacity for chemical modification [17, 32]. The high surface area of CNF makes it a good candidate for the production of aerogels.

CNF aerogels can be functionalized chemically for different applications by post-treatment of formed aerogels or by modification of precursor nanofibers prior aerogel production. For example, titanium dioxide (TiO_2) nanoparticles [33] and silanes [34–36] have been used to make superhydrophobic materials via post-treatment. As a result, wetting of the aerogel is prevented while oil can be adsorbed into the pores of the aerogel (Figure 3). The oil could be removed by exposure to organic solvents enabling multiple uses of the aerogel. Modification of precursor nanofibers with particle coating is a facile way to prepare a material of interest as oil-adsorbent for sustainable applications. Additionally, deposition of titanium dioxide on CNF aerogels makes them a photosensitive hybrid material [37]; in fact, the ability of TiO_2 nanoparticles to respond to UV light can switch the wetting behavior of the aerogel from hydrophilic to hydrophobic [37].

CNF-based aerogels have also been studied as environmentally friendly alternatives to replace superabsorbent polymers (SAP). CNF aerogels absorb water at high rates; however they typically have half the water loading capacity of conventional SAPs. Aerogels also have weaker mechanical stability in the wet state relative to that when dry. Two approaches have been used to improve this property: (1) addition of macroscopic fibers as reinforcement [38], and (2) crosslinking to obtain aerogels with fast shape recovery upon water sorption [39]. Conductivity of these aerogels can be affected by polyaniline–dodecyl benzene sulfonic acid treatment [31], and incorporation of magnetic domains can be used to generate magnetic properties allowing large stimuli-responsive deformation [40]. Modification with 2,2,6,6-Tetramethylpiperidine 1-oxyl (TEMPO) produces aerogels with excellent

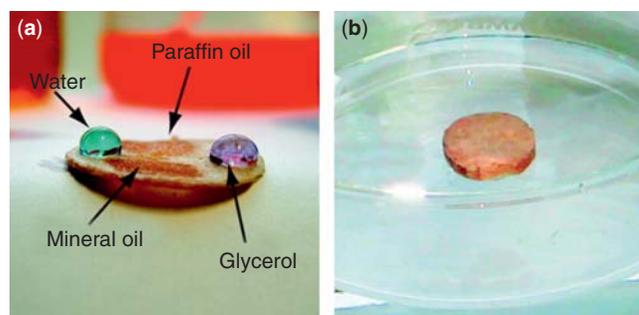


Figure 3 Superhydrophobic and oleophilic CNF aerogel produced by loading it with titanium dioxide nanoparticles. The hydrophobic and oleophilic properties are indicated by the fact that water (colored with reactive blue dye) and glycerol droplets are not absorbed by the CNF- TiO_2 -coated aerogels. However, paraffin oil and mineral oil droplets are readily absorbed (a). The TiO_2 -coated aerogels float on water (b). Reprinted with permission from [33]. Copyright (2011) American Chemical Society.

catalytic efficiency for the azide-alkyne Huisgen cycloaddition reaction, suggesting that fibrillar aerogels are also suitable supports for catalysts [41].

In addition to the aerogel structures described above, paper-like films of CNF, nanopaper, prepared via removal of water by supercritical drying, liquid CO_2 evaporation or freeze-drying can be considered as CNF aerogels. These systems are porous membranes with potential use in fuel cells, catalysis, liquid purification and filtration, tissue engineering, protein immobilization, protein separation, protective clothing, etc. [42]. Overall, there is a tremendous potential in tailoring the functions of CNF aerogels upon chemical modifications, making this an active area of current research.

3 CNF-BASED NANOCOMPOSITES

Historically natural fibers have been used in composites primarily for reinforcement. Several applications of natural fibers in the manufacture of composites for the automotive [43–46] and other industries are available [47–49]. In automotive applications natural fibers offer several advantages over glass fiber: they have lower cost, are lightweight, biodegradable, and less abrasive to processing equipment. However the recyclability, moisture repellence/sensitivity, durability and flame retardancy are still some issues that need to be addressed for implementation on a large scale [43, 50]. Fiber reinforced composites have been used in a variety of interior car parts such as door trim panels, door liners, headliners, cabin linings and seatbacks [43, 45]. However, car exterior components present obvious challenges.

Compatibility of the fibers with polymers is one of the key issues that have been largely investigated. The main challenge appears to be the poor adhesion and dispersibility of the fibers in the hydrophobic polymer matrices due to the dissimilar nature of the components. In fact, several physical and chemical modifications to improve fiber-matrix adhesion have been suggested [51–55], including silane coupling [56]. The inherent properties of the source natural fibers, such as, structure, mechanical and thermal properties, can have a large impact in the manufacture process and their suitability for composites [55, 57–60].

Cellulose has been proven to have great potential as reinforcing material in thermoplastics and elastomers [61]. Improved adhesion between cellulosic fibers and polypropylene was reported when the fibers have been accomplished upon pretreatment with silane coupling agents in methanol/water solution and also with maleic anhydride modified polypropylene (MAPP) [62]; however, this procedure leads to thermal

degradation of the cellulosic fibers during processing. The nature of the adhesion between cellulose and polypropylene in composites has been studied [63] by treating the cellulosic fibers first with toluene in a Soxhlet system during 24 h and then dried, followed by immersion in a solution of 5% maleic anhydride modified polypropylene in toluene. The dried fibers and polypropylene were then mixed in a mixing extruder for further processing by injection molding. The results indicated highly hydrophobic fibers upon pretreatment (contact angle $\sim 140^\circ$) that led to an improved interfacial adhesion resulting in increased composite mechanical properties (tensile modulus and strength at yield). Additionally, a pre-activation of the MAPP at 180°C allows to increase the reactivity of the polymer that reacts with the hydroxyl groups at the surface of cellulose forming strong, ester covalent bonds that improve adhesion with polypropylene [63]. Another way to increase the hydrophobicity of fibers is by graft copolymerization of methyl acrylate; in this case the composites display improved mechanical properties [64].

As with natural fibers, surface modification of CNF is critical for incorporation in composite material. Several reports have dealt in detail with the development of nanocomposites [23, 65–67], the inclusion of nanofibers in composites [24, 50, 68–72], and the resultant barrier properties of the nanocomposites [6]. Approaches as those used in the case of cellulose fibers can also be applied for CNF. For example, acetylation, silane coupling (silylation), graft copolymerization, treatment with isocyanate, triazine or physical treatments including electrical discharge (corona and cold plasma) and mercerization [51, 54, 55]. Areas of research in cellulose nanofiber composites include applications in food packaging, automotive industry, construction materials, furniture production, transparent films for displays, nanopaper, and filtration membranes. The production of such nanocomposites includes simple methods such as casting aqueous CNF dispersions using water soluble matrix materials; casting films from CNF dispersions mixed with polymer latex dispersions; surface modification of CNF followed by mixing in a solvent with the polymeric matrix to cast films; dispersion of dry CNF into the hydrophobic matrix, etc. [73]. Following are examples of the application of CNF in composites.

3.1 CNF in Packaging Films

CNF barrier properties are important in packaging applications, a topic that has received extensive attention in the literature [6, 17, 74–78]. For example, comparison of the barrier properties of cellulose nanocrystals and microfibrillated cellulose from sisal

fibers showed that water diffusion was higher for films made from CNCs compared to CNF [79]. Mass transport properties of CNF films have been evaluated where films were produced with glycerol as plasticizer [76]. Glycerol reduces the water uptake at low humidity whilst the opposite effect occurred at higher humidity; the diffusion coefficient was higher for plasticized samples, despite the porosity of the films they showed high oxygen barrier properties. Nanocomposite films of TEMPO-oxidized nanocellulose/montmorillonite presented high tensile strength and low oxygen permeability at 0 and 50% relative humidity, these properties were ascribed to the hydrogen bonding between the TEMPO oxidized cellulose and the montmorillonite [80]. Pulp characteristics and treatments used to produce the cellulose microfibrils play an important role in the resultant barrier properties [6].

3.2 Optically Transparent Composites

CNF has been studied for possible applications in flexible display manufacture [12]. Films ($\sim 60\ \mu\text{m}$ thickness) from Douglas fir CNF obtained by grinding method gave low light transmittance as a dried sheet, but transparency increased after polishing the film [13]. BC has also been used to prepare transparent cellulose nanocomposites manufactured by using different resins, epoxy, acrylic and phenol formaldehyde (Figure 4a) [12]. The fiber content in the BC nanocomposites (up to 70% CNF content) have very low thermal expansion coefficient. Compared to the neat epoxy resins the BC-epoxy nanocomposite displayed 10% less light transmittance (Figure 4b). Transparent films ($\sim 20\ \mu\text{m}$ thick) have been produced by suction filtration of TEMPO-oxidized hardwood and softwood CNF (Figure 5) [81]. Other approaches to produce transparent films include acetylation of cellulose fibrils and further graft polymerization with hydroxyethyl methacrylate (HEMA) to produce a continuous hydrogel matrix of poly(2-hydroxyethyl methacrylate (PHEMA) [82]. Electrically conductive transparent paper was made by incorporation of fibroin solution into BC membrane followed by adsorption of multi-walled carbon nanotubes [83].

3.3 Nanopaper

Nanopaper has been a subject of recent studies due to its properties, such as low thermal expansion coefficient, optical transparency and good mechanical properties combined with a simple preparation procedure (filtration from aqueous suspensions) [84]. The typical preparation of nanopaper is carried out by vacuum filtration of CNF suspensions [85] to produce films of different porosities and thickness ($\sim 70\text{--}90\ \text{nm}$).

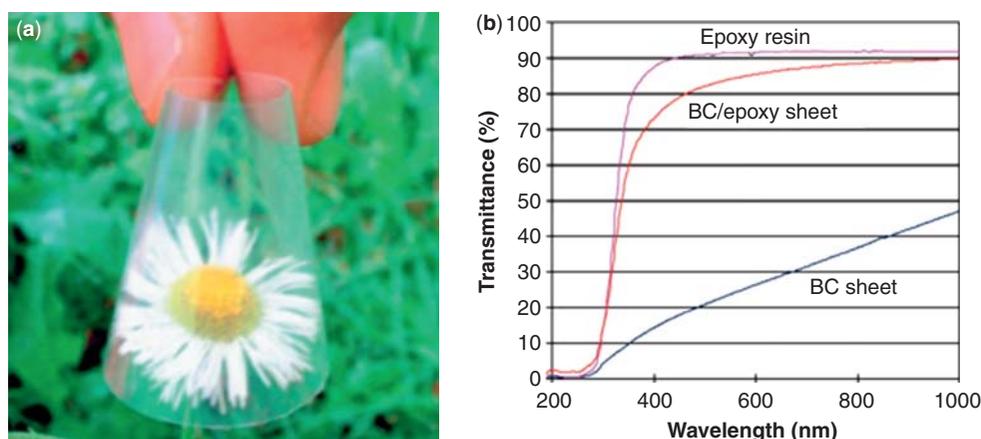


Figure 4 Image of a 65 μm transparent and flexible CNF film (a). Transmittance of raw materials and nanocomposite at different wavelengths (b). Reprinted with permission from [12]. Copyright (2005) John Wiley and Sons.

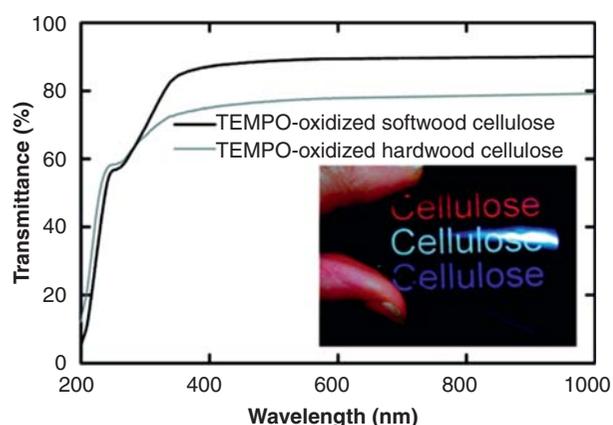


Figure 5 Transmittance (UV-vis) of softwood CNF films prepared by TEMPO oxidation. Reprinted with permission from [81]. Copyright (2009) American Chemical Society.

Yield stress of around 90 MPa has been measured. The mechanical strength can be improved by using carboxymethylated fibers prior to fibrillation (Young modulus of 13.2 GPa and tensile strength of 214 MPa) [85].

In addition to bleached birch, nanopaper from unbleached birch pulp has been prepared in order to evaluate the effect of residual lignin on the mechanical properties. Higher density was observed for the unbleached fibrils compared to that for fully bleached fibrils. High air barrier properties were observed for the nanopaper from unbleached fibers as well as better mechanical properties (tensile index and stress at break), although elastic modulus was lower (9 GPa) than nanopaper from fully bleached fibers (11 GPa). High water absorption (~ 4 times higher) was observed for paper produced from unbleached nanofiber compared to fully bleached fibers, this effect was explained by the presence of heteropolysaccharides and the effect of mechano-radicals during the fibrillation process [86].

An attempt to obtain nanopaper with preferred fiber orientation was reported by Sehaqui *et al.* [84]. TEMPO-oxidized nanofibers were used to produce nanopaper by using cold drawn technique. In this technique a wet cake is produced by vacuum filtration of the suspension and then cut in strips that are stretched at a defined rate until the desired strain is reached (20, 40 and 60%). Once stretch is stopped, the samples were dried under vacuum at 93°C for 12 minutes. The mechanical properties of the nanocomposites increased linearly with the strain applied (draw ratio), with a modulus increase from 10 GPa (non-drawn) up to 33 GPa (60% strain). AFM images of the nanopapers are presented in Figure 6.

The factors that affect the elastic modulus of nanopaper have been studied [87]. By using never-dried sulfite pulp pretreated by TEMPO-oxidation prior to fibrillation, and using numerical modelling, the authors were able to identify the main factors that affect the elastic modulus of nanopaper. Results indicated that drying constraints did not affect the elastic properties of the nanopaper. It was also found that the amorphous regions along the length of the nanofibers affected the properties of the nanopaper. The behavior of the nanopaper at high strain was evaluated by modelling a reduction in contacts (bonds) in the fiber network: it was found that a decrease in 60% of the contacts produces a reduction of 25% in the elastic modulus for the 3D network [87].

Recently in a comparative study of paper and nanopaper prepared from BC and CNF of canola straw [88], a reduction of crystallinity was observed due to the grinding process used to produce the nanofibers. In terms of thermal properties, the CNF nanopaper showed a reduction in onset point compared to BC nanopaper. Films prepared from both canola cellulose nanofibers and BC displayed high barrier properties to air and

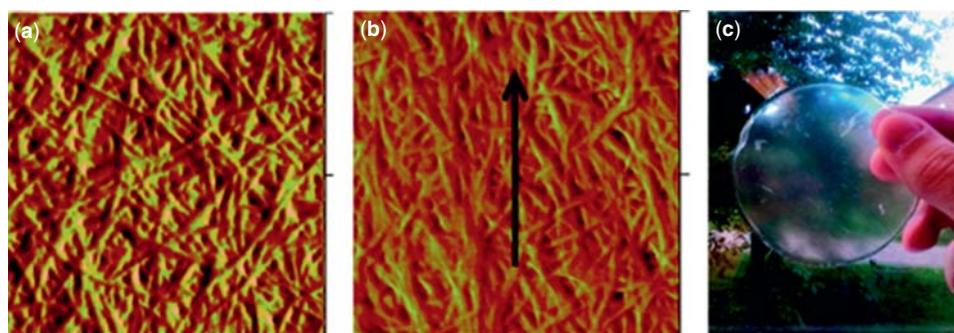


Figure 6 Reference non-drawn (a) and drawn to 40% strain (b) TEMPO CNF nanopaper (arrow showing the orientation of the fibers). Self-standing nanopaper is shown in (c). Reprinted with permission from [84]. Copyright (2012) American Chemical Society.

good mechanical strength, with Young modulus of 13.6 and 17.3 GPa for CNF and BC nanopaper, respectively.

In addition to strength and air barrier, fire retardant properties of clay-filled nanopaper have been investigated [89]. Clay is typically used in polymeric composites. Unlike the traditional approach to disperse clay particles in a polymer solution enabling the polymer to penetrate the interlayers leading to intercalation or exfoliation of the particles [90–94], the fibrils are not able to enhance clay exfoliation. The clay and the fibrils are proposed to form a fibril network enclosing groups of clay particles within the pores (Figure 7) [89]. This considerably increases the importance of the dispersion stability prior to nanopaper or composite formation. It has been shown that by controlling the nanoclay dispersion media its affinity towards CNF can be tuned [95]. Using the platy clay particles in a suitable electrolyte medium, a thin, densely packed layer can be formed on the substrate. The interaction between the particles and the fibrils can be tuned depending on if one phase dispersion or a layered structure is constructed. Both traditional clay grades [96] as well as nanosized particles [89, 97] have been used in preparation of clay-CNF hybrid materials.

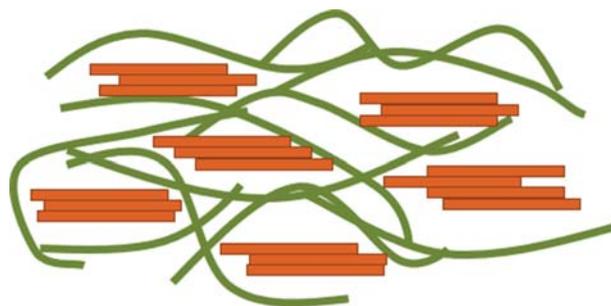


Figure 7 A proposed structure of cellulose nanofibrils and clay particles. Reprinted with permission from [89]. Copyright (2011) American Chemical Society.

The cellulose fibrils make the matrix holding the film together and giving it strength, while the clay particles improve moisture resistance as well as flame retardancy [85]. Fifty percent clay loading in the nanocomposite produced a paper with high ultimate strength (124 MPa), which was ascribed to the structural characteristics of clay platelets and its orientation within the composite. The composite nanopaper that could withstand indicated a high modulus (8.7 GPa) after thermal exposure at 200°C compared to 11 GPa at room temperature [89].

4 CNF-BASED BIOACTIVE MATERIALS

Biocompatibility, high hydrophilicity, high surface area, and good mechanical strength [17, 32], makes CNF an ideal candidate for fabrication of several biomaterials. In this section a few examples are highlighted regarding: (1) biosensors and diagnostics; (2) pharmaceuticals; (3) cell culture and tissue engineering; (4) antimicrobial agents and wound dressings.

4.1 Biosensors and Diagnostics

CNF is a suitable platform for immobilization of bioactive molecules (e.g., enzymes, antibodies, etc.), which is useful in biosensors and diagnostics. For example, novel gold nanoparticle (NP)-bacterial cellulose (Au-BC) nanocomposites have been prepared by a facile one-step biotemplated method in aqueous suspension [98]. Due to its excellent biocompatibility, good conductivity, and ultrafine nanofiber network structure, Au-BC nanocomposites can easily entrap horseradish peroxidase (HRP) while still maintaining enzyme bioactivity. HRP biosensors allow detection of H_2O_2 with a detection limit lower than 1 μM . CNF platforms are not limited to HRP, and may be applicable to many enzymes with different functionalities.

Surface functionalized CNF films have been developed for biosensors and diagnostics [99]. As shown in Figure 8 (Method I), CNF-films were first carboxylated with (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO), then the surface was activated via EDC/NHS coupling chemistry. Next, antibody (antihuman immunoglobulin G (anti-IgG)) was immobilized on the sensor either by physical adsorption or ink-jet printing. Carboxylation treatment enhances the water resistance of the CNF-film, resulting in a robust biointerface, while the increased hydrophilicity results in low nonspecific binding. Moreover, the developed CNF-based surface can detect positively charged molecules, which in many cases is challenging.

By using similar surface activation methods (TEMPO-mediated oxidation and carboxylation), Avidin has been conjugated on the activated CNF film for selectively capturing biotinylated molecules as shown in Figure 8 (method II) [100]. After binding biotinylated anti-IgG, these modified CNF films were found effective in detecting human IgG.

Recently, our group proposed a novel sensor based on peptide decorated CNF film for specific detection of human IgG (unpublished work). Instead of antihuman IgG, the HWRGWVA peptide which has specific binding affinity to IgG was used, as proposed elsewhere [101]. Acetylated peptide was covalently immobilized to the spacers on CNF via the amide reaction. Spacers with amine groups were prepared by two different

methods: First, a random copolymer, poly (2-aminoethyl methacrylate hydrochloride-co-2-hydroxyethyl methacrylate) (poly(AMA-co-HEMA)) was grafted from initiator-modified CNF substrates by surface initiated polymerization. Second, the amine containing chitosan was physically adsorbed onto the CNF. The developed peptide-modified CNF sensor surfaces were characterized by a number of techniques, and their specific binding affinity for target IgG as well as capability to minimize nonspecific proteins adsorption were determined by quartz crystal microgravimetry (QCM). It was found that by using chitosan as spacer in the sensor, very high specific binding capability of IgG is obtained while exhibiting excellent resistance to nonspecific protein adsorption.

Commercial silver particles have been used in CNF nanocomposites [102] and antimicrobial paper was synthesized following approaches reported in disposable biosensors [103] and filtering materials [104]. Similar systems based on BC responded to light from fluorescent silver nanoclusters (Figure 9) [104]. More details about antibacterial applications will be addressed in other sections of this review.

4.2 Pharmaceuticals

Cellulose and its derivatives have a long history in pharmaceutical technology, mainly as an excipient in oral solid dosage forms [106]. It is suggested that

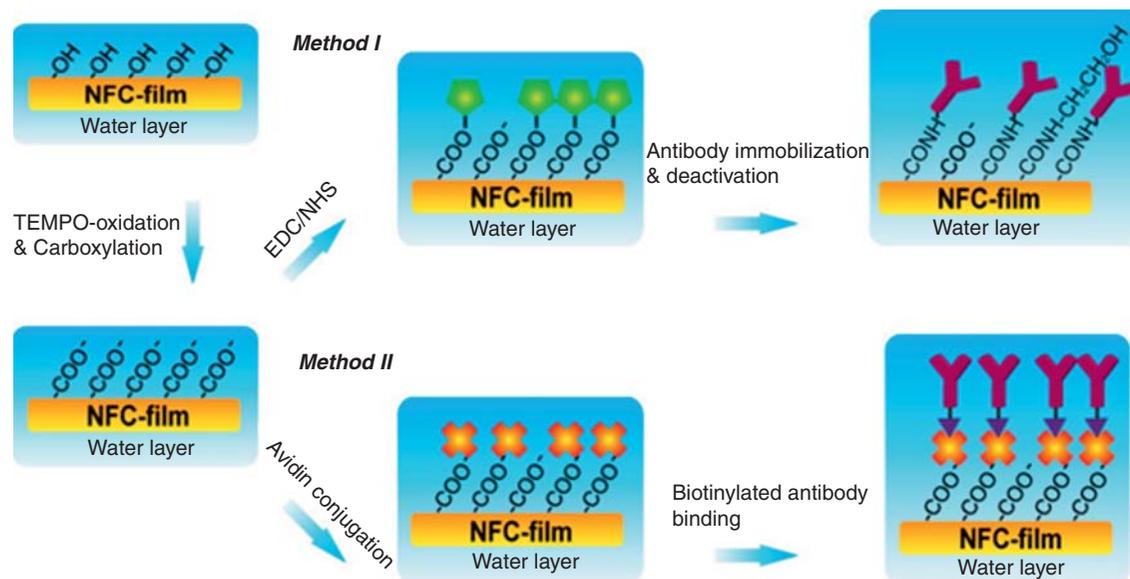


Figure 8 Schematic illustration of the immobilization of antibodies on the modified CNF-films after TEMPO-mediated oxidation and carboxylation. Method I: the pretreated CNF-films were activated by EDC/NHS to generate NHS groups for covalent conjugation of antibodies via amide chemistry. Adapted from [99]. Method II: the pretreated CNF-films were conjugated by Avidins following by binding of biotinylated antibodies. Adapted with permission from [100]. Copyright (2012) American Chemical Society.

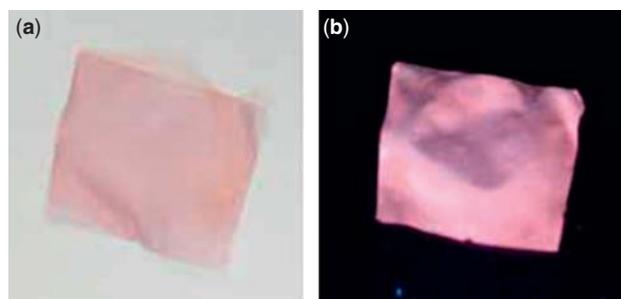


Figure 9 CNF nanopaper functionalized with fluorescent silver nanoclusters under white (a) and UV-light (b). Reprinted with permission from [105]. Copyright (2011) John Wiley & Sons, Inc.

CNF could be used to form macroscopic structures that act as templates for stabilization of nanoparticles [40], providing a potential practical application in pharmaceuticals. For example, a drug delivery system based on CNF matrix was developed recently [107]. Nanoparticles of Itraconazole (a hydrophobic drug) were stabilized effectively by the nanostructured cellulose matrix during freeze-drying and storage. In addition, the dissolution rate of Itraconazole was increased significantly as a consequence of the formation of the immobilized nanodispersion, which also enhanced the *in vivo* performance of the drug [107]. Other researchers have produced novel tableting materials using spray-dried CNF in order to increase the long-term stability of drugs [108]. The results showed that compared with commercial microcrystalline cellulose, CNF-based excipients have a better ability to pack with lower powder porosity. Furthermore, CNF tablets disintegrate slightly faster, which causes faster drug release from direct compression tablets [108].

Besides its application as a stabilizing agent and excipient, CNF can also be used for drug loading and controlled release. For example, BC membranes have been investigated as systems for topical or transdermal drug delivery [109, 110]. Lidocaine was used as a model anesthetic drug; the uniform distribution of the drug inside the BC membranes was observed and the *in vitro* diffusion studies showed that the incorporation of Lidocaine in BC membranes provided lower permeation rates than those obtained with the conventional delivery systems (e.g., aqueous solutions and gels). Moreover, this technology can be successfully applied to modulate the bioavailability of drugs for percutaneous administration [110]. The greatest advantage of BC membrane is the combination of its wound healing capacity and the ability to absorb exudates with the release of therapeutically relevant drugs, which could be particularly advantageous in the design of new delivery systems or application in mucosa and epidermis.

4.3 Cell Culture and Tissue Engineering

Due to their excellent mechanical strength, high water content, ultrafine, highly pure nanofibril network structure and its inherent biocompatibility, CNF is suitable for the applications of tissue engineering. Several examples of such applications for CNF or BC can be found in the literature. For example, BC has been evaluated as a potential scaffold for tissue-engineered blood vessels (TEBV), and its interaction with smooth muscle cells (SMC) has been reported [111]. The asymmetric structure composed of a fine network of nanofibrils is similar to a collagen network, which promotes the adhesion and proliferation of SMC on the BC pellicle [111]. An effort to replace part of the carotid artery (4–6 mm) of a rat using BACTERIAL SYNTHESIZED CELLULOSE (BASYS[®]) has been reported in the literature [112]. After four weeks, the carotid-artery-BASYS[®] complex was wrapped up with connective tissue and was infused with small vessels, showing that BC can be used as a replacement for blood vessel [112]. The response of primary bovine chondrocytes on BC has also been examined [113]. The mechanical properties of unmodified BC were found to be comparable or better than those of tissue culture plastic and alginate, while they showed significantly higher levels of chondrocyte growth at similar levels of *in vitro* immune response; these facts suggest the potential application of scaffolds for cartilage tissue engineering [113].

Bhattacharya *et al.* demonstrated that plant-derived native CNF hydrogel could be used as a 3D cell culture [114]. CNF was injectable due to small viscosity at high shear stress and its rheological characteristics allowed adequate mixing of cells into the gel. The spontaneously formed gel after injection can provide mechanical support for cell growth and differentiation. Beneficial properties of CNF are based on its unique nanofibrillar structure mimicking properties of the extracellular matrix (ECM). Especially, it was shown that a single component CNF scaffold promotes hepatocyte 3D cell culture without added bioactive components, which is an advantage from the immunological point of view.

Immobilization of functional proteins or peptides is a widely used strategy to control and guide the interactions between cells and materials [115]. For example, the functionalization of BC surfaces with recombinant proteins containing a bioactive peptide (IKVAV) and a carbohydrate-binding module (CBM3) improved their biocompatibility, as well as that for neuronal and mesenchymal cells [116]. In order to improve the blood compatibility and regenerative ability of BC, BC nanofibrils should be crosslinked with some materials to prevent the formation of blood clots. This problem has been addressed with the use of heparin, a typical anticoagulant agent for modification. The

cell compatibility results indicated that the modified BC nanofibers were suitable for cell proliferation and ingrowth [117]. To mimic the natural collagen fibers, a natural peptide, ϵ -polylysine (PLL) was introduced to the surfaces of BC nanofibers via crosslinking method [118]. The BC/PLL nanofibers proved to have similar shape and molecular structure compared with natural collagen nanofibers. After immersion in CaCl_2 and simulated body fluid, the BC/PLL nanofibers served as nanotemplates to induce the formation of nano-sized platelet-like, calcium-deficient, B-type carbonated hydroxyapatite (HAp). The final mineralized BC/PLL nanofibers exhibited similar architecture and composition of natural bone, suggesting that BC/PLL nanofibers are promising biomaterials in bone tissue engineering.

CNF can also be used to form a nanocomposite material for cell culture [119]. For example, nanocomposites of BC networks and calcium-deficient HAp have been produced by introducing the mineral phase into the bacteria culture medium during formation of cellulose fibrils. The biocompatibility and cell viability were confirmed by HEK cell seeding, making this nanocomposite potentially viable in biomedical applications. In another report, biocomposite scaffolds consisting of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P(3HB-co-4HB)) and BC were prepared, which combined their respective advantages [120]. The resultant scaffolds exhibited 3D network structure composing of multi-distribution of pore size and proved to be capable of forming cell adhesion and proliferation.

4.4 Antibacterial Agent and Wound Dressing

Another application of CNF in the biomedical field is their use as antibacterial agent and wound dressing. A widely used method is to integrate silver nanoparticles to CNF because silver metal and its compounds have been known for their strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities [121, 122]. The assembly of the CNF and silver nanoparticle (AgNP) composites can be carried out through electrostatic interaction of AgNP (aqueous colloids) onto CNF using polyelectrolytes as linkers [102]. Their antibacterial activity against *S. aureus* (gram-positive bacteria) and *K. pneumoniae* (gram-negative bacteria) was reported [102]. Another strategy is through magnetic interaction [123]. Here, the unique, ultrafine 3D structure of the BC matrix can provide plenty of sites for heterogeneous nucleation of magnetite. The resultant magnetic Ag nanocomposite possesses a high antimicrobial activity against the model microbes *E. coli* (gram-positive

bacteria) and *Bacillus subtilis* (gram-negative bacteria). The combination of magnetic nanoparticles with silver nanoparticles in the nanostructure of BC makes it an interesting approach to develop recoverable and reusable antibacterial agents [123]. Finally, the functionalization of native CNF using silver nanoclusters (AgNC) to form a novel type of functional nanocellulose/nanocluster composite was found to exhibit a pronounced antibacterial activity [105]. Instead of preformed particles, silver nitrate was used to precipitate silver by reducing the ions to metallic nanoparticles using sodium borohydride. The CNF/AgNC composite retained the appealing properties of both components. As observed in Figure 10, the bacteria grew freely all around the unmodified CNF film, indicating no antibacterial activity. In contrast, a large halo free of bacteria was observed around the CNF/AgNC films due to the release of silver ions or AgNC, which prevented bacterial growth. These results suggested this CNF/AgNC composite may find application in wound-healing pads.

BC is an interesting material for using as a wound dressing since it can control wound exudates and can provide moist environment to a wound resulting in better wound healing [124]. Biofill® is a commercial product of BC used as a temporary substitute for human skin in cases of second- and third-degree burns [112, 125]. In order to prevent wound infection AgNP have been incorporated in the BC membranes [11, 126, 127]. Maneerung *et al.* impregnated AgNP into bacterial cellulose through chemical reduction [11]. The modified BC exhibited a strong antimicrobial activity against *S. aureus* and *E. coli*, which are bacteria found in the contaminated wounds. Especially, the

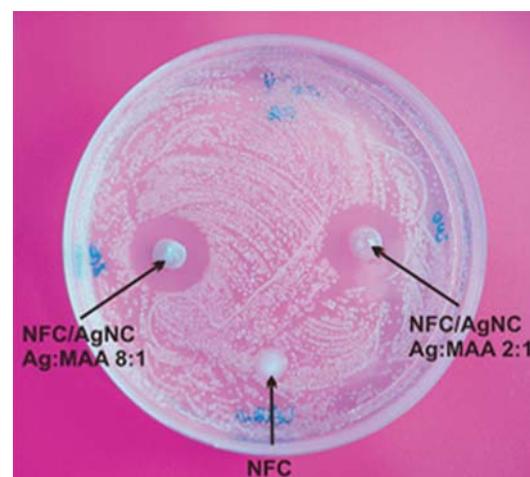


Figure 10 Antibacterial properties of CNF films functionalized with silver nanoclusters. Reprinted with permission from [105]. Copyright (2011) John Wiley & Sons, Inc.

impregnation, instead of coating the BC with AgNP, improved the antimicrobial activity of the BC and lowered the possibility of normal human tissue damage.

Besides AgNP, quaternary ammonium compound is another group of well-known antibacterial agents. Furthermore, cellulose has been covalently grafted with a quaternary ammonium compound octadecyldimethyl (3-trimethoxysilylpropyl) ammonium chloride (ODDMAC) by a simple adsorption-curing process [128]. The resultant films showed very good antibacterial capacity even at very low concentrations of antimicrobial agent immobilized on the surface. Because ODDMAC is covalently immobilized onto the material surface, this film exhibits long-term antimicrobial activity. Moreover, due to the large surface area to volume ratio of the cellulose films and the fact that each fibril is modified with ODDMAC, these nonporous films could be very effective as antimicrobial separation filters.

There is great potential for the use of CNFs in a range of applications. So far their applications in aerogels, composites and the biomedical field have been discussed. Some examples of inorganic/organic hybrids have been mentioned in these sections: TiO₂ (aerogels), clay (nanocomposite section), and silver (bioactive materials). In the next section of this review other important CNF-inorganic hybrids will be introduced.

5 CELLULOSE NANOFIBRIL HYBRIDS

Hybrid materials combine organic and inorganic components aiming for synergistic effects. An increasing number of studies presenting the combination of cellulosic nanofibrils with inorganic particles are being reported. The inorganic phase is typically added to improve the barrier and optical properties, as well as to enable preparation of responsive materials with the incorporation of functional particles.

5.1 Silica

Silica particles are extensively used in a variety of applications for optical effects as well as for providing durability, for example, in surface finishes. Mainly for these reasons silica nanoparticles have been used for creating hybrid structures with cellulose fibrils. For instance, a hybrid structure of bacterial cellulose with silica nanoparticles was prepared using tetraethoxysilane as a precursor. High loading of up to 66% was achieved with the particles attached to the fibrils [129].

Another approach for preparing a bacterial cellulose-silica network by growing the fibrils in a silica dispersion using the bacteria has been presented [12]. The bacterium (*Ga. Xylinus*) was noted for being

more active in acid conditions leading to loadings as high as 50%. The concentration of the silica solution was found to affect the amount of silica in the structure formed. Bacterial growth in a silica matrix is a simple alternate approach to enable silica particles to penetrate into a preformed cellulose matrix. However, the loading could not be increased over ca. 10%. The fibril layer in the silica hybrid structures provides strength whereas the silica brings the functionality. However, with high silica loadings the strength of the matrix is compromised. It can be predicted that hybrid materials combining cellulose nanomaterials and silica will make their way into (electronic) displays and related technologies.

5.2 Responsive Hybrid Materials

There is a growing interest in hybrid materials able to respond to external stimuli. Here we present a few examples of the most common types of responsive materials involving the incorporation of UV active and magnetic particles into the CNF network, enabling preparation of responsive cellulosic products. Needless to say, such materials have promising potential in packaging and sensing.

In a study leading to preparation of conductive BC network with photoswitchability, the photosensitivity was created by introducing vanadium nanoparticles on a cellulosic self-standing film [130]. Addition of these particles altered the film, i.e., changed the color of the nanopaper to green. This effect was further altered by photoactivation via irradiating the sample leading to color change to purple (Figure 11). The change in color was due to changes in the oxidation stage of the vanadium. Quite importantly to sensing materials the effect is reversible and the film can be used multiple times. In addition to vanadium, a photosensitive CNF hybrid material has been prepared by deposition of titanium dioxide film on CNF aerogels [37], already discussed in the aerogels section of this review.

Magnetic cellulose materials offer solutions for separation techniques and filtering as well as in intelligent packaging. Producing cellulosic fibrils with magnetic characteristics offers opportunities in many applications where magnetic components are already used, such as biomedical and separation techniques [131, 132]; the organic phase can be used to provide functionality to the magnetic domains. For example, as discussed earlier, embedding magnetic domains into a cellulose aerogel can be used to prepare super-adsorbent structures that can be triggered with applied magnetic fields [40]. In the case of cellulose matrix structured as BC the hybrid material can be deployed as a magnetic scaffold [133]. The most

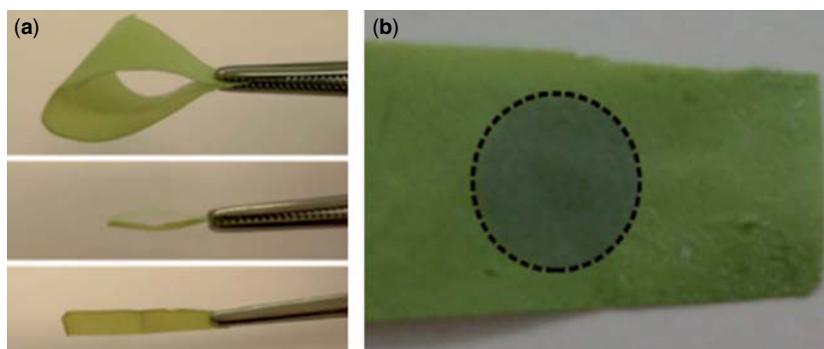


Figure 11 Hybrid materials developed from vanadium-containing cellulose displaying photoactive effects. Images of the foldable green vanadium oxide nanopaper are shown in (a). Changes in color are observed after irradiation of the green vanadium oxide nanopaper (dashed circle in (b)). Reprinted with permission from [130]. Copyright (2012) John Wiley & Sons, Inc.

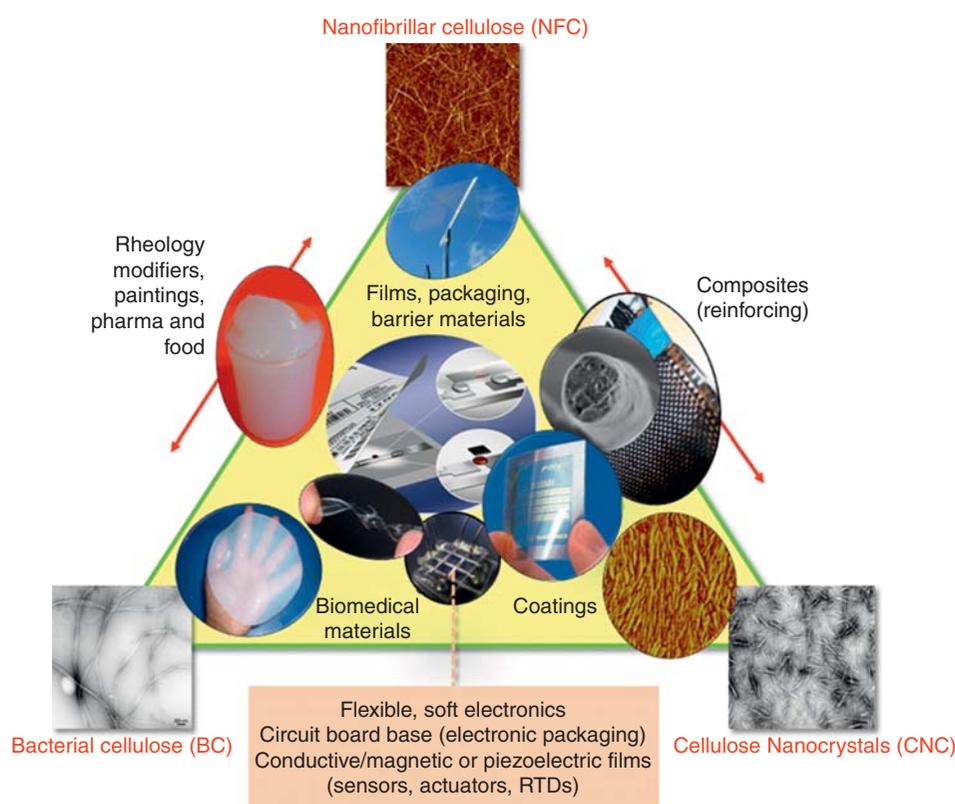


Figure 12 Nanocellulose (BC, CNF and CNC) (triangle apex) and some of the current applications (sides of the triangle). The second generation materials to be developed from the knowledge base in this project are also indicated (center of the triangle); this includes hybrid materials for fluidics, electronics and sensing applications.

efficient way to increase a high loading in the cellulosic matrix appears to be by precipitation of the particles using precursor salts.

6 FINAL REMARKS

In this review we highlighted some uses of cellulose nanofibrils after deconstruction of fibers in plants or from its assembly by microorganisms. Wood and

non-wood plants are abundant, renewable, and sustainable and thus they represent a natural resource of incredible importance. It has been recognized that nanocellulose in three of its forms, cellulose nanofibrils (CNF), cellulose nanocrystals (CNC) and bacterial cellulose (BC), have properties and attributes that can be suited for different applications and for creating new materials. While this review only concentrated on the fibrillar forms of cellulose (CNF

and BC), together with CNC these materials offer a number of opportunities for developments in different fields, as illustrated in Figure 12. A major part of research activities has concentrated on the utilization of CNF in traditional high volume, low cost products such as paper, packaging, paints, composites and food (some of which were not covered in this review). In these areas of application, removal of water in nanocellulose dispersions is a challenge that demands the development of methods for surface functionalization of fibrils to improve dewatering. Such efforts seem to progress at a relatively slow pace. There is also an opportunity to address the critical need to innovatively develop new platforms that will lead to another generation of nanocellulose-based products. This can be achieved by using the power of nanoscience along with a full understanding of the behavior (e.g., structural assembly) of different types of nanocellulose; by characterizing and exploiting their surface chemistry and interactions with other materials at the nanoscale; and by exploring several of its properties (structural, physical, chemical, thermal, piezoelectric, etc.) (Figure 12).

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