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Feature Article

Nanocellulose in biomedicine: Current status and future prospect

Ning Lin, Alain Dufresne *

Grenoble Institute of Technology (Grenoble INP), The International School of Paper, Print Media and Biomaterials (Pagora), CS10065, 38402 Saint Martin d'Hères Cedex, France

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ABSTRACT

Nanocellulose, a unique and promising natural material extracted from native cellulose, has gained much attention for its use as biomedical material, because of its remarkable physical properties, special surface chemistry and excellent biological properties (biocompatibility, biodegradability and low toxicity). Three different types of nanocellulose, viz. cellulose nanocrystals (CNC), cellulose nanofibrils (CNF) and bacterial cellulose (BC), are introduced and compared in terms of production, properties and biomedical applications in this article. The advancement of nanocellulose-based biomedical materials is summarized and discussed on the analysis of latest studies (especially reports from the past five years). Selected studies with significant findings are emphasized, and focused topics for nanocellulose in biomedicine research in this article include the discussion at the level of molecule (e.g. tissue bioscaffolds for cellular culture; drug excipient and drug delivery; and immobilization and recognition of enzyme/protein) as well as at the level of macroscopic biomaterials (e.g. blood vessel and soft tissue substitutes; skin and bone tissue repair materials; and antimicrobial materials). Functional modification of nanocellulose will determine the potential biomedical application for nanocellulose, which is also introduced as a separated section in the article. Finally, future perspectives and possible research points are proposed in Section 5.

Contents

* Corresponding author. Tel.: +33 476826995; fax: +33 476826933.
E-mail address: Alain.Dufresne@pagora.grenoble-inp.fr (A. Dufresne).

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1. What is nanocellulose? – Types and productions

With the emergence and development of nanotechnology, cellulose, the most ancient and important natural polymer on earth revives and attracts more attention in the new form of "nanocellulose" to be used as novel and advanced material. Nanocellulose is described as the products or extracts from native cellulose (found in plants, animals, and bacteria) composed of the nanoscaled structure material. Generally, the family of nanocellulose can be divided in three types, (1) cellulose nanocrystals (CNC), with other designations such as nanocrystalline cellulose, cellulose (nano) whiskers, rod-like cellulose microcrystals; (2) cellulose nanofibrils (CNF), with the synonyms of nanofibrillated cellulose (NFC), microfibrillated cellulose (MFC), cellulose nanofibers; and (3) bacterial cellulose (BC), also referred to as microbial cellulose [1,2].

The sources for CNC and CNF extraction are wood, cotton, hemp, flax, wheat straw, sugar beet, potato tuber, mulberry bark, ramie, algae, and tunicin. As shown in Fig. 1 (top images), the production of CNC or CNF is a procedure consisting in converting the large unit (cm) to the small unit (nm). Chemically induced destructuring strategy, such as acid hydrolysis, is commonly performed for the extraction of CNC from native cellulose, through the removal of amorphous regions and preservation of highly-crystalline structure. Released nanoparticles (CNC) present a diameter of 5–30 nm, and length of 100–500 nm (from plant cellulose), or length of 100 nm to several micrometers (from tunicate and algae celluloses). With microscopic observations and light scattering techniques, the morphology and dimensions of CNC can be assessed as elongated rod-like (or needle-like) nanoparticles, and each rod can therefore be considered as a rigid cellulosic crystal with no apparent defect [3].

Regarding the preparation of CNF, mechanically induced destructuring strategy is mainly applied, which involves high-pressure homogenization and/or grinding before and/or after chemical or enzymatic treatment. Multiple mechanical shearing actions can effectively delaminate individual microfibrils from cellulosic fibers. Different from rigid CNC, CNF consists of both individual and aggregated nanofibrils made of alternating crystalline and amorphous cellulose domains, which attributes the morphology of CNF with soft and long chains. Due to the entanglement of long cellulosic chains, it is not so easy to determine the length of CNF (commonly regarded as higher than 1 μm) with microscopic techniques. Therefore, only the information of fibril width for CNF is generally provided in the studies, which varies from 10 to 100 nm depending on the source of cellulose, defibrillation process and pretreatment [4].

Contrary to the production of CNC and CNF, the biosynthesis of BC is a process of construction from tiny unit (Å) to small unit (nm). As shown in Fig. 1 (bottom images), BC is typically synthesized by bacteria (such as *Acetobacter xylinum*) in a pure form which requires no intensive processing to remove unwanted impurities or contaminants such as lignin, pectin and hemicellulose. During the biosynthesis of BC, the glucose chains are produced inside the bacterial body and extruded out through tiny pores present on the cell envelope. With the combination of glucose chains, microfibrils are formed and further aggregate as ribbons (nanofibers) [11]. These ribbons subsequently generate a web-shaped network structure with cellulosic fibers (BC), which has a diameter of 20–100 nm with different types of nanofiber networks.

It is crucial to discuss the issue of large-scale production of nanocellulose, which determines the practical applications of nanocellulose as available commercial products. According to the reports of "Future Markets Inc." [12], a number of organizations have announced CNF and CNC demonstration plants in Europe and North America. It seems that the countries in North America focus on the production of CNC, such as reported organizations of Bio Vision (Canada), CelluForce (Canada) and US Forest Service Forest Products Laboratory (USA); while European countries are more interested in CNF, for instance reported organizations of Centre Technique du Papier (France), Stora Enso (Finland), UPM Fibril cellulose (Finland), Borregaard Chemcell (Noway), etc. In comparison with the large-scale production of CNC and CNF, the production of BC is rather limited, resulting from high cost to support the growth of bacteria and low yield. Despite numerous bioreactors that have been studied in the literature to produce BC on large

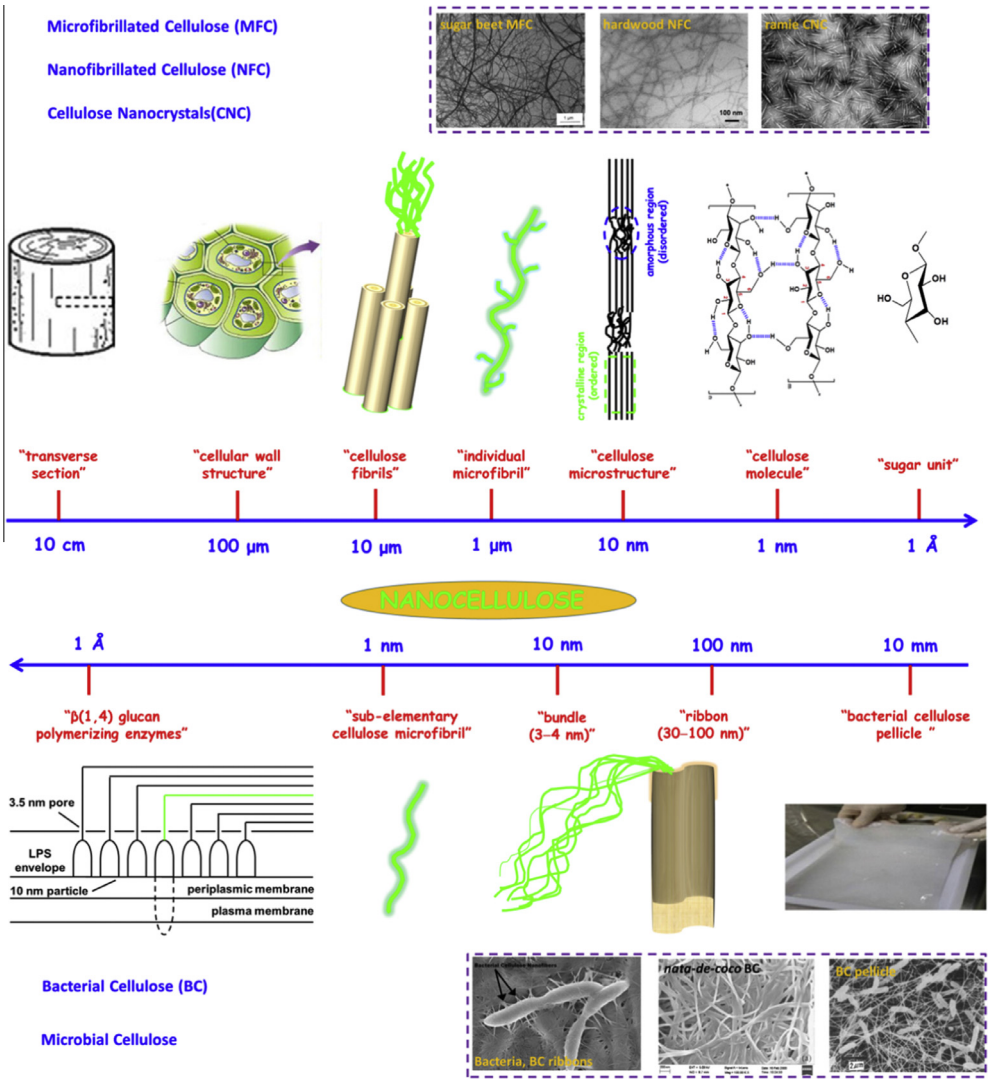

Fig. 1. Hierarchical structure of cellulose; top image (from large unit to small unit): cellulose nanocrystals (CNC), micro/nanofibrillated cellulose (MFC and NFC); bottom image (from tiny unit to small unit): bacterial cellulose (BC). Transmission electron micrographs of sugar beet MFC (adapted with permission from [5]), hardwood MFC (adapted with permission from [6]), ramie CNC (adapted with permission from [7]); and scanning electron micrographs of BC ribbons (adapted with permission from [8]), *nata-de-coco* BC (adapted with permission from [9]), BC pellicle (adapted with permission from [10]).

scale, the highest BC productivity is only 0.38 g/(L h) with the aerosol bioreactor [13]. The organization of Jenpolymers (Germany) and Nutrasweet Kelco Company (USA, with the trade name of Primacel in the 1990s) were ever reported to produce BC and related biomedical products.

2. Why the choice of nanocellulose? – Unique properties in physics, chemistry and biology

As natural nanoscaled material, nanocellulose possesses diverse characteristics different from traditional materials, including special morphology and geometrical dimensions, crystallinity, high specific surface area, rheological properties, liquid crystalline behavior, alignment and orientation, mechanical reinforcement, barrier properties, surface chemical reactivity, biocompatibility, biodegradability, lack of toxicity, etc. On the basis of these unique properties, both "nano-enhanced" and completely new "nano-enabled" products have been envisioned ranging from bulk applications like rheological modifier, composite reinforcement or paper additive, to high-end applications such as tissue engineering, drug delivery and functional material [14]. All the properties of nanocellulose can be generally classified in three parts, viz. physical properties, surface chemistry, and biological properties. Associated with the topic "biomedicine", the emphasis of this article is mainly placed on the mechanical reinforcement, surface groups and charges, as well as various biological properties of nanocellulose.

2.1. Mechanical properties and potential nanoreinforcement

The mechanical properties of nanocellulose can be characterized by its properties in both the ordered (crystalline)

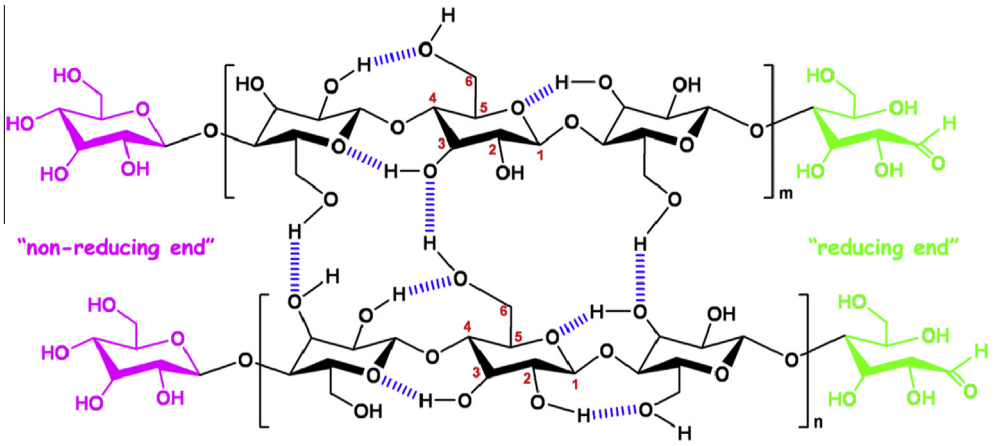

Fig. 2. Schematic representation of the chemical structure and intra-, inter-molecular hydrogen bonds in crystalline cellulose.

and disordered (amorphous) regions of the nanoparticle. Cellulosic chains in disordered regions contribute to the flexibility and plasticity of the bulk material, while those in ordered regions contribute to the stiffness and elasticity of the material. The modulus of different types of nanocellulose is expected to result from a mixing rule between the modulus of the crystalline domains and the amorphous fraction. Therefore, the stiffness and modulus of CNC with more crystalline regions should be higher than those of CNF and BC fibrils with both crystalline and amorphous structures.

Since 1930s, the elastic modulus of crystalline cellulose has been investigated either by theoretical evaluations or by experimental measurements (wave propagation, X-ray diffraction, Raman spectroscopy, and atomic force microscopy). A broad range of values was reported, and it is generally accepted that the Young's modulus of crystalline cellulose (assimilated to the one of CNC) should be in the range 100–200 GPa, which gives specific values similar to Kevlar (60–125 GPa) and potentially stronger than steel (200–220 GPa). Recently, the elastic modulus of crystalline cellulose was investigated from atomistic simulations using both the standard uniform deformation approach and a complementary approach based on nanoscale indentation, which was reported as 139.5 ± 3.5 GPa (similar to Kevlar) [15]. In another study, Dri et al. performed the atomic structure model of cellulose in tandem with quantum mechanics to compute the Young's modulus of crystalline cellulose, which predicted the modulus of crystalline cellulose as high as 206 GPa (similar to steel) [16].

Again, a broad range of values for the longitudinal modulus of cellulose microfibrils (involving both CNF and BC) was reported based on different theoretical and experimental strategies. The accepted average value is around 100 GPa for the modulus of cellulose microfibril. A three-point bending experiment using atomic force microscopy tips was performed on cellulose microfibrils to calculate the elastic modulus. The dimension of cellulose microfibrils was found to significantly affect the mechanical properties, and a value of 81 ± 12 GPa was reported to be the longitudinal modulus of pulp CNF [17]. Recently, the modulus of BC was reported as 114 GPa through the analysis of a Raman spectroscopic technique, which involved the determination of local molecular deformation of BC via a shift in the central position of the 1095 cm^{-1} Raman band [18].

Originated from these impressive mechanical properties, nanocellulose has been potentially used as a load-bearing element for various host materials. With the homogenous dispersion and strong interfacial adhesion, the presence of high-modulus nanocellulose can exhibit the promising nanoreinforcement allowing proper stress transfer from host material (matrix) to the reinforcing phase (nanocellulose).

2.2. Surface chemistry

From a structural point of view, cellulose is a high molecular weight homopolysaccharide composed of β-1,4-anhydro-D-glucopyranose units (Fig. 2). These units do not lie exactly in the plane with the structure, but rather they assume a chair conformation with successive glucose residues rotated through an angle of 180° about the molecular axis and hydroxyl groups in an equatorial position [19]. The ability of these hydroxyl groups to form hydrogen bonds plays a major role in the formation of fibrillar and semicrystalline packing, which governs the important physical properties of this highly cohesive material [20]. As indicated with blue dashed lines in Fig. 2, intramolecular hydrogen bonds occur primarily between the hydrogen borne by the OH group of the C3 carbon and ring oxygen of the adjacent glucose unit (O5). The intermolecular hydrogen bonds occur between the hydrogen of the OH–6 primary hydroxyl and oxygen in position O3 in a cycle of a neighboring unit, as well as the hydrogen of OH–2 and oxygen in position O6.

It is well known that the unidirectional parallel orientation of cellulose chains within the elementary fibrils, occurring during biosynthesis and deposition, induces the formation of crystals having hydroxyl functionality on one end, known as the non-reducing end (shown in pink[1]

[1] For interpretation of color in Fig. 2, the reader is referred to the web version of this article.

in Fig. 2), and hemiacetal functionality on the other, known as the reducing end (shown in green in Fig. 2).

One of the most specific characteristics of cellulose is that each of its glucose unit bears three hydroxyl groups, which endows nanocellulose a reactive surface covered with numerous active hydroxyl groups. For each anhydroglucose unit, the reactivity of hydroxyl groups on different positions is heterogeneous. The hydroxyl group at the 6 position acts as a primary alcohol whereas the hydroxyl groups in the 2 and 3 positions behave as secondary alcohols. Indeed, the carbon atom which carries the hydroxyl group in the 6 position is only attached to one alkyl group, while the carbons with the hydroxyl groups in the 2 and 3 positions are joined directly to two alkyl groups, which will induce steric effects derived from the supramolecular structure of cellulose and the reacting agent [21]. It has been reported that on the structure of cellulose, the hydroxyl group at the 6 position can react ten times faster than the other OH groups, while the reactivity of the hydroxyl group on the 2 position was found to be twice that of at the 3 position [22]. However, regarding the surface reactivity of hydroxyl groups from nanocellulose (such as CNC), the use of reactants or solvents may affect the reactivity of hydroxyl groups from different positions. Recent studies reported the order of reactivity for hydroxyl groups on CNC as nucleophiles with OH-C6 = OH-C2 > OH-C3 by etherification [23,24].

Apart from reactive groups, another important issue for the surface chemistry of nanocellulose is the surface charges, which mainly refers to the negative sulfate esters ($-OSO_3^-$) on CNC. Surface sulfate esters are introduced on CNC during sulfuric acid hydrolysis via condensation esterification (sulfation) between surface hydroxyls and a H_2SO_4 molecule, using another H_2SO_4 molecule as a condensation agent. The H_2SO_4 hydrolyzed CNC, therefore, are highly negatively charged, and form a well-dispersed aqueous colloidal suspension. Surface charge amount from sulfate groups on CNC can be controlled through the duration and temperature of H_2SO_4 hydrolysis. Besides the promotion of high stability of CNC in solvents, surface $-OSO_3^-$ groups with negative charges also provide CNC the accessibility for biomedical application, such as electrostatic adsorption of enzymes or proteins [25].

2.3. Biological properties

2.3.1. Biocompatibility and hemocompatibility

Biocompatibility is referred to as the ability of a foreign material implanted in the body to exist in harmony with tissue without causing deleterious changes, which is an essential requirement for biomedical materials [26]. Regarding the evaluation of cellulose biocompatibility, different studies provide various results due to the range of methodologies and sample preparations. According to the early reports [27,28], cellulose can be generally considered to be broadly biocompatible, invoking only moderate (if any) foreign body responses *in vivo*. However, it is well known that cellulose is not readily degraded by the human body because it lacks cellulolytic enzymes, which will inevitably cause some incompatibility. It is a pity that direct investigations on the biocompatibility of CNC and CNF are rare. Some studies on CNC-based materials (such as hydrogels) only report experiments of cell cultivation, through the growth, propagation and activity of cells to evaluate the conditions of material biocompatibility. Diverse CNC-based materials as bioscaffolds for cell cultivation will be further discussed in following section. Hemocompatibility (or blood compatibility) is another significant property of biocompatibility, especially for blood-contacting biomaterials and artificial organs, such as artificial blood vessels, pumps, and artificial hearts. Interestingly, recent study reported the regulation of blood metabolic variables by the presence of TEMPO-oxidized cellulose nanofibers. The oral administration of TEMPO-oxidized cellulose nanofibers to mice was proved to be effective for reducing the postprandial blood glucose, plasma insulin, glucose-dependent insulinotropic polypeptide, and triglyceride concentrations. It seems that TEMPO-oxidized cellulose nanofibers have both promising hemocompatibility and unique biological activities [29].

Attributed to its biosynthesis procedure, BC is commonly regarded as a material possessing better biocompatibility than other types of nanocellulose. With an *in vivo* study of subcutaneous BC implantation in rats for 12 weeks [30], no fibrotic capsule or giant cells were found, indicating no foreign body reaction for the introduction of BC in animals. Meanwhile, fibroblasts infiltrated BC, which was well integrated into the host tissue, did not elicit any chronic inflammatory reactions [30]. Gama et al. investigated the biocompatibility of small-diameter BC and peptide (Arg-Gly-Asp)-modified BC membranes subcutaneously implanted in sheep for 1–32 weeks. Compared with negative control samples [expanded polytetrafluoroethylene (ePTFE)], peptide-modified BC membranes were only mildly irritating to the tissue, with no significant differences in the inflammation degree [31]. In another study, *in vivo* biocompatibility of the BC membrane was analyzed through histological analysis of long-term subcutaneous implants in mice. BC implants caused a mild and benign inflammatory reaction that decreased with time and did not elicit a foreign body reaction. Moreover, no differences were observed between the controls and implanted animals in thymocyte populations and in B lymphocyte precursors and myeloid cells in the bone marrow [32]. With the plasma recalcification time and whole blood clotting experiments, Gama et al. studied the hemocompatibility of BC and BC-based biomaterials. It was reported that native BC and peptide (Arg-Gly-Asp)-modified BC membranes both preserved original conformational structures and exhibited a favorable interaction (non-activation) with platelets, which indicated BC and modified BC as promising hemocompatible biomaterials [33]. Similar conclusions were recently reported for the hemocompatibility study of BC/polypyrrole [34] and BC/polyvinyl alcohol biocomposites [35].

2.3.2. Biodegradability in vivo

For some applications (e.g. artificial heart valves or menisci), biocompatible, non-biodegradable materials may be acceptable whereas for other applications (e.g. artificial bone grafts), the bioresorbable material enabling tissue regeneration is preferable [36]. In terms of

biodegradation, cellulose may be considered as non-biodegradable *in vivo* or, at best, slowly degradable, due to the lack of cellulase enzymes in animals. However, the form (i.e. crystallinity, hydration and swelling) of cellulose may affect the degree of degradation, absorption and immune response. Nonenzymatic, spontaneous biodegradability of cellulose chains may perhaps account for slow breakdown of unaltered cellulose within the human body, though this is admittedly conjecture and has not been adequately studied [37]. In an early *in vivo* study, Miyamoto et al. found that the degradation of cellulose and cellulose derivatives in canine specimens depended significantly on the cellulose crystalline form and chemical derivatization [27]. Regenerated cellulose prepared by deacetylation of cellulose acetate (presumably the highly crystalline cellulose II polymorph) did not measurably degrade over the course of the 6-week experiment. Contrarily, however, up to 75% (w/w) of equivalent samples of amorphous regenerated cellulose were degraded and absorbed over the same experimental period. Another study reported that CNC was actually more biodegradable than fullerenes and carbon nanotubes in aqueous environments, but without the *in vivo* investigation of biodegradability [38]. Recently, oxidized cellulose was rendered more vulnerable to hydrolysis and therefore potentially degradable by the human body. Based on this strategy, researchers attempted to enhance the biodegradability of nanocellulose through oxidation, such as the report of improving BC degradability *in vitro* (in water, phosphate buffered saline, and simulated body fluid) through periodate oxidation [39,40]. With the pre-γ-irradiation and sodium periodate oxidation treatments on BC membranes, it was reported that *in vitro* degradation of oxidized BC involved two major phases, (1) initial rapid degradation of about 70–80% of the entire sample; (2) slower degradation of an additional 5–10% which eventually levels off leaving a small amount of non-resorbable material. Further experiments on *in vivo* degradation (male New Zealand White rabbits) showed the marked degradation of oxidized BC membranes at all-time points, with the most rapid degradation occurring in the first 2–4 weeks [41].

2.3.3. Toxicology

Even though earlier studies have reported nanocellulose to have no or low toxicity (comparable to that of table salt), when used as biomedical materials, the issues of toxicology and safety concerns for these natural nanomaterials should be further emphasized. Since the beginning over twenty years ago, the nanotoxicology research for nanoparticles has built a comprehensive assessment system, such as for metallic nanoparticles (Au, Ag nanoparticles, quantum dot, etc.) and carbon nanotubes. However, the toxicology study of nanocellulose and nanocellulose-based biocomposites is still restricted at a very preliminary stage (mainly on the level of cytotoxicity). Table 1 summarizes recent reports on toxicology experiments and conclusions for nanocellulose. On the whole, there is no evidence for serious influence or damage of nanocellulose on both cellular and genetic level as well as *in vivo* organ and animal experiments. However, the inhalation of plentiful nanocellulose (especially for CNC) may induce pulmonary inflammation due to the easy self-aggregation and non-degradation of nanocellulose in the body of animals.

Kovacs et al. initially studied the inherent eco-toxicology of cellulose nanocrystals with aquatic organisms (different species of fish) [42]. Rainbow trout hepatocytes were selected as the model cells, and the toxicity monitoring program as well as the in-depth toxicity assessment component was included in the toxicity testing strategy. With the eco-toxicological characterization, CNC was found to have low toxicity potential and environmental risk, but showed no harm to aquatic organisms at concentrations that could occur in receiving waters. In another report, the cytotoxicity of CNC against nine different cell lines was determined both by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay and lactate dehydrogenase (LDH) assay, and no cytotoxic effects of CNC against any of these cell lines in the concentration range and exposure time studied (0–50 μg/mL and 48 h) were reported [46]. However, recently it was reported that CNC may induce some slight dose-dependent cytotoxic and inflammatory effects on human lung cells, especially the risk with inhalatory exposure under high concentrations of released CNC powders [43].

Regarding the toxicity of cellulose nanofibrils, no inflammatory effects or cytotoxicity on mouse and human macrophages, and only low acute environmental toxicity (assessed with kinetic luminescent bacteria test) have been reported [47]. VTT Technical Research Centre in Finland proposed an evaluation report on the systemic study of CNF for *in vitro* cytotoxicity, genotoxicity, immunotoxicity, and neurotoxicity, together with pharyngeal aspiration study on mice. The results revealed low cytotoxicity and no DNA or chromosome damage from CNF, but pulmonary inflammation for mouse experiment possibly induced by the particulate/bacteria from CNF [49,50]. Pereira et al. evaluated the *in vitro* cytotoxicity and the effect on gene expression of CNF to fibroblasts cells. It was reported that low concentrations of CNF (100 μg/mL) have no obvious toxicity, whereas high concentrations of CNF (2000 and 5000 μg/mL) will cause the sharp decrease of cell viability and affect the expression of stress- and apoptosis-associated molecular markers [52]. Alexandrescu et al. compared the cytotoxicity on fibroblast cells of pure CNF and surface modified-CNF with crosslinking agent polyethyleneimine (PEI) and surfactant cetyl trimethylammonium bromide (CTAB). In comparison with no acute toxic phenomena for pure CNF, both modified-CNF samples caused a significant reduction in cell viability and proliferation [51]. Interestingly, in another recent study, cationic modified-CNF (trimethylammonium-CNF) was reported to display a better cytocompatibility than unmodified and anionic modified-CNF (carboxymethylated-CNF) [53].

Attributed to biosynthesis procedure during the preparation, bacterial cellulose is commonly regarded as one of the most biocompatible material in the family of nanocellulose. As shown in Table 1, no cytotoxicity for BC was obtained according to the evaluation on osteoblast cells, endothelial cells, and mouse feeding experiment [54–56].

Although studies conducted so far on nanocellulose reported the absence of serious environmental and biological concerns, research and systematic assessment of

Table 1
Toxicological evaluations of nanocellulose.

Type	Toxicological experiment	Conclusion	Ref.
CNC	• Acute lethal test • Multi-trophic assays • Animal experiments with fathead minnow and *Zebrafish* reproduction tests • *In vitro* rainbow trout hepatocyte assay	• Low toxicity potential • Low environmental risk	[42]
	• Respiratory toxicity of aerosolized CNC on the human airway with a co-culture of human monocyte-derived macrophages, dendritic cells and a bronchial epithelial cell line	• Low cytotoxicity • Somewhat ***(pro-)inflammatory cytokines***	[43]
	• *In vitro* gene mutations • *In vitro* and *in vivo* chromosomal tests • Skin irritation and sensitization tests • Animal experiments with rat feeding study (28 d)	• No evidence of high toxicity	[44]
	• Cytotoxicity evaluation with L929 cells	• Low cytotoxicity at low CNC concentration	[45]
	• Cytotoxicity evaluation with nine different cell lines	• No cytotoxic effects in the concentration range (0–50 μg/mL) and exposure time (48 h)	[46]
CNF	• Cytotoxicity evaluation with human monocyte and mouse macrophages • Kinetic luminescent bacteria test for acute environmental toxicity	• No evidence of inflammatory effects or cytotoxicity	[47]
	• *In vitro* genotoxicity with enzyme comet assay	• No significant DNA damage	[48]
	• Neurotoxicity and systemic effects with a nematode model	• Low or no cytotoxicity	[49,50]
	• *In vitro* pharyngeal aspiration study for pulmonary immunotoxicity and genotoxicity with mice	• No DNA and chromosome damage • ***Pulmonary inflammation***	
	• Cytotoxicity evaluation with 3T3 fibroblast cells (including the test of cell membrane, cell mitochondrial activity and DNA proliferation)	• No toxic phenomena for pure CNF • Somewhat ***cytotoxicity for modified-CNF*** (with PEI or CTAB surface modification)	[51]
	• Cytotoxicity evaluation with bovine fibroblasts cells • Effects of gene expression *in vitro*	• Low cytotoxicity at low CNF concentration (0.02–100 μg/mL) • Reduction of cell viability and affection of the expression of stress- and apoptosis-associated molecular markers at high CNF concentration (2000–5000 μg/mL)	[52]
	• Cytotoxicity evaluation with human dermal fibroblasts	• No evidence of cytotoxicity for pure CNF • Improved cytocompatibility of EPTMAC-modified CNF	[53]
BC	• Cytotoxicity evaluation with osteoblast cells and L929 fibroblast cells	• No evidence of cytotoxicity	[54]
	• Cytotoxicity evaluation with human umbilical vein endothelial cells • Animal experiment with C57/Bl6 male mouse	• No evidence of toxicity *in vitro* and *in vivo*	[55]
	• *In vitro* immunoreactivity with human umbilical vein endothelial cells • *In vivo* intraperitoneal injection study with BALB/c male mice	• Non-toxicity and non-immunogenicity	[56]

Abbreviations: PEI, polyethyleneimine; CTAB, cetyl trimethylammonium bromide; EPTMAC, glycidyltrimethylammonium chloride.

eco-toxicology of nanocellulose still need deeper investigations, especially aimed to the effects and mechanisms of nanoparticles aggregation in the body, and long-term *in vivo* toxicity evaluation of nanocellulose. Moreover, not only the toxicity of nanocellulose itself, what the toxicity effects will be induced by the incorporation of nanocellulose is another important issue, which indicates the eco-toxicology of nanocellulose-based materials. Despite no significant cytotoxicity of nanocellulose-based materials (generally hydrogels) in many studies [57–60], there was also a report of negative effect on biocompatibility for nanocellulose-based composites [61].

3. Nanocellulose-based biomedical materials

The development of novel biomedical materials from natural polymers for practical and clinical applications is always a most concerned topic for biologists and material scientists. In some studies, information on nanocellulose and its application are mentioned as the term "biocellulose," which is attributed to the unique properties and potential of nanocellulose in the study of diverse biomedical materials. According to the report of "Future Markets Inc." in "The global market for nanocellulose to 2017" published in October, 2012, there will be about $ 97 billion estimates for medical and life sciences markets impacted by nanocellulose [12]. It is the aim of this section to discuss the research in biomedical application of nanocellulose with selected latest examples. From the molecular level (cellular cultivation) to macroscopic biomaterials (drug delivery, substitute implants, tissue repair, regeneration, etc.), diverse studies and new frontiers together with future strategies on nanocellulose-based biomedical materials are highlighted and remarked.

3.1. Tissue bioscaffolds for cellular culture

Attributed to the properties of biocompatibility and right mechanical properties similar to natural tissue, nanocellulose-based biomaterials can provide a cell-friendly environment to encourage cells attachment and proliferation as a special tissue bioscaffold. Diverse cellular species cultured on nanocellulose-based biomaterials have been reported, and the forms of these materials include hydrogels, composites, electrospun nanofibers, sponges, and membranes. BC seems to be the most prevalent choice for the medium of cells culture because of its low cytotoxicity and high porosity.

Regarding CNC-based media for the culture of cells, some studies applied conventional suspensions of unmodified or fluorescent-modified nanocrystals as the environment for cells. From insect cells S*f*9 and Hamster lung fibroblast V79, to human foreskin fibroblasts, human embryonic kidney cells HEK-293 and human lung cell, no significant cytotoxicity to various cell models was found, and promising cellular uptake and proliferation were reported in these studies [43,62–64]. Using a spin-coating method, Dugan et al. prepared submonolayer film with oriented surfaces of adsorbed CNC. Due to the shape and nanoscale dimensions of CNC, murine myoblasts cells C2C12 adopted increasingly oriented morphologies in response to more densely adsorbed and oriented nanocrystals surface. With a mean feature height of only 5–6 nm, CNC surface presented the smallest features to induce contact guidance in skeletal muscle myoblasts [65,66]. Recently, electrospun nanofibers based on CNC, bearing suitable mechanical property, *in vitro* degradation and basic cytocompatibility, were proved to be promising bionanocomposite scaffolds for cell culture. It was reported that electrospun maleic anhydride-grafted poly(lactic acid) nanofibers reinforced with CNC can be the supporting scaffolds to culture the human adult adipose derived mesenchymal stem cells (hASCs) and promote cell proliferation. Low CNC concentration effectively improved the thermal stability and mechanical properties of scaffolds, but not significantly caused any cytotoxic effect on hASCs proliferation within 7 days [67]. Another study reported the application of all-cellulose scaffold materials (CNC/cellulose) to culture cells, and the influence of CNC orientation in scaffolds to cell growth was investigated. Fig. 3(a and a′) presents confocal laser scanning microscope (CLSM) images of Human dental follicle cells (hDFCs) cultured on electrospun CNC/cellulose nanofibers for 3 and 7 days. It was shown that CNC can well dispersed in electrospun scaffolds and achieve considerable orientation along the long axis direction. Cultured cells can proliferate rapidly not only on the surface but also deep inside the scaffolds. More interestingly, the aligned nanofibers of CNC/cellulose exhibited a strong effect on directing cellular organization [68].

Under controlled concentrations, CNF aqueous suspensions can spontaneously form hydrogels to provide suitable environment with required mechanical support for cell growth and differentiation. It was reported that CNF hydrogels can promote the cellular differentiation of the human hepatic cell lines (HepG2 and HepaRG), and induced the spheroid formation of cells [69]. A novel scaffold composed of natural polymers was reported to culture NIH3T3 fibroblast, involving the components of pectin, carboxymethyl cellulose and CNF [70]. Recently, highly porous and biomimetic nanocomposites that allow for modulating the growth of L-929 fibroblasts were prepared by incorporating calcium peroxide (CaO_2) and catalase into CNF matrix. Fig. 3(b and b′) shows CLSM images of L-929 fibroblasts cultured on pristine CNF and CNF modified with 15 wt% calcium peroxide/catalase composite scaffolds. The addition of CaO_2 and catalase induces the presence of hydrogen peroxide (H_2O_2) or oxygen (O_2), which affected the survival of cells. Three-dimensional porous morphology of CNF-based scaffolds both facilitated the diffusion of generated gases and provided great niches for cell growth. It was reported that due to the generation of H_2O_2, cell attachment decreased, and cell proliferation was delayed; while the generation of O_2 played a useful role in supporting cell proliferation [71].

Various BC-based bioscaffolds for the application of cellular culture can be mainly divided into three aspects, which are BC pellicle/membrane scaffolds, BC/matrix biocomposite scaffolds, and surface-modified BC pellicle scaffolds. Several latest studies focused on pure BC as scaffolds for supporting cellular adhesion and proliferation. Favi et al. investigated BC as a hydrogel scaffold for the culture of equine-derived bone marrow mesenchymal stem cells (EqMSCs). As shown in Fig. 3(c and c′), BC hydrogels were cytocompatible, and significantly supported cellular adhesion and proliferation. The cells seeded on the BC hydrogel were observed to be viable and metabolically active [72]. Park et al. investigated the alteration of function of human umbilical vein endothelial cells treated with α,β-unsaturated aldehyde on BC scaffold. The study indicated that α,β-unsaturated aldehydes in cigarette smoke induce altered endothelial cell functions including morphology, adhesion, proliferation, viability, and growth on BC [73]. Another work presented a cellular building unit made from microstrand-shaped BC covered with mammalian cells. By folding or reeling the building unit, the multiple shapes of millimeter-scale cellular constructs (coiled and ball-of-yarn-shaped structures) were investigated. Histological analysis of the cellular constructs indicated that the BC microstrand served as a pathway of nutrition and oxygen to feed the cells in the central region [74]. Recently, the laser patterning post-processing was used on BC to solve the limitation of small and heterogeneous pore size for the ingrowth of cells. After laser perforation, BC hydrogels displayed high biocompatibility and the resulting channels supported migration, matrix production and phenotypic stabilization of bovine chondrocytes, which qualified perforated-BC as a sustainable scaffold for cell ingrowth [75].

Regarding the materials used for the BC/matrix scaffold systems, chitosan, agarose, alginate, collagen, gelatin, polypyrrole, and poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-co-4HB)] have been studied as matrices to culture cells. Polypyrrole was in situ polymerized onto the surface of BC to produce the BC/polypyrrole membrane scaffold, and performed the seeding of PC12 rat neuronal cells. Conductive polypyrrole coating on BC acted as an

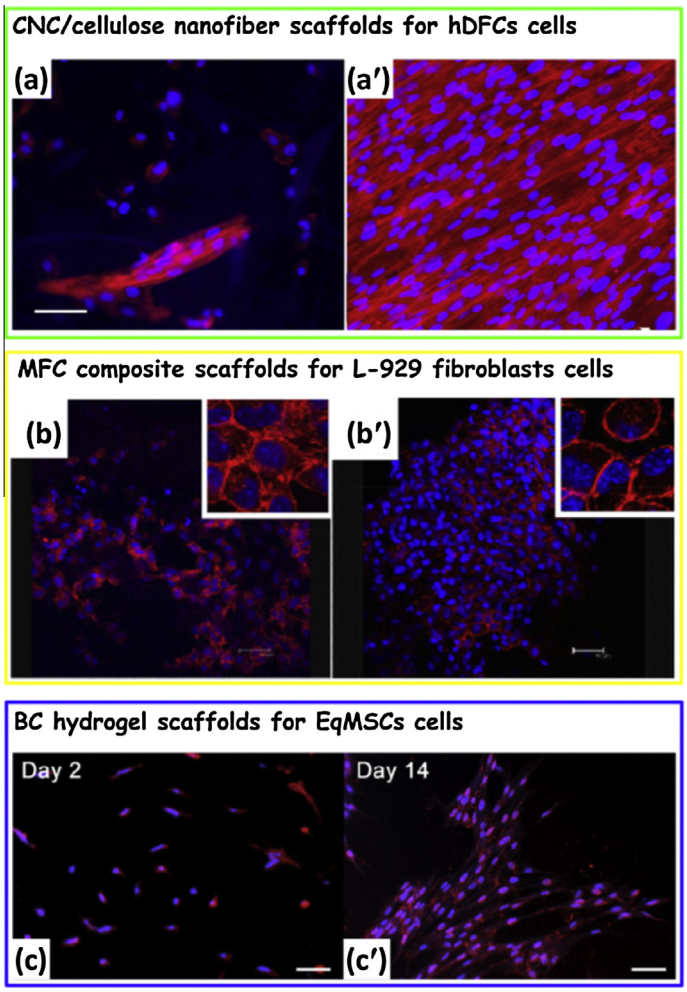

Fig. 3. Confocal laser scanning microscope (CLSM) images of nanocellulose bioscaffolds for cell culture. Human dental follicle cells (hDFCs) cultured on electrospun CNC/cellulose nanofibers for (a) 3 days, (a′) 7 days; scale bar = 50 μm (adapted with permission from [68]). L-929 fibroblasts cultured for 7 days on (b) pristine CNF, (b′) CNF/15 wt% calcium peroxide/catalase composite; scale bar = 40 μm (adapted with permission from [71]). Equine-derived bone marrow mesenchymal stem cell (EqMSCs) cultured on BC hydrogels for (c) 2 days, (c′) 14 days; scale bar = 50 μm (adapted with permission from [72]).

active interface for tissue engineering, which was beneficial for the regulation of cell activity through electrical stimulations [76]. Incorporated with gelatin or hydroxyapatite (Hap) to enhance the bioactivity, Wang et al. developed a porous BC membrane with regular vertical pore arrays via a laser patterning technique. Chondrogenic rat cells were cultured on these membrane scaffolds, and the scaffolds well supported the attachment and proliferation of cells together with the preservation of cellular viability [77]. Another study prepared BC-biocomposite scaffolds by freeze-drying using polymeric P(3HB-co-4HB) as matrix and trifluoro-acetic acid as co-solvent. Chinese hamster lung fibroblast cells were incubated on this composite scaffold for 48 h, which exhibited the capability of cell adhesion and proliferation, as well as better biocompatibility than pure P(3HB-co-4HB) scaffold [78].

To further enhance cell attachment on BC-based bioscaffolds, surface modifications can be performed, such as protein or peptide coatings, plasma treatment, surface sulfation or phosphorylation. Shi et al. prepared a BC scaffold coated with bone morphogenetic protein-2 (BMP-2). The alkaline phosphatase activity assays indicated that BC had a good biocompatibility and induced the differentiation of mouse fibroblast-like C2C12 cells into osteoblasts in the presence of BMP-2 *in vitro*. Within a certain range (0–3 mg/scaffold), the osteogenic activity of induced osteoblasts was positively correlated to the concentration of BMP-2. *In vivo* subcutaneous implantation studies further showed that BC scaffolds modified with BMP-2 promoted more bone formation and higher calcium concentration than the BC scaffolds alone at 2 and 4 weeks [79]. Another surface modification strategy using plasma treatments (O_2, N_2, or CF_4 plasmas) on BC reported altered changes of surface property involving more hydrophilic BC with O_2 or N_2 plasma treatment, and hydrophobic BC with CF_4 plasma treatment. Furthermore, different surface plasma treatments on BC scaffolds will provide distinct effects for the adhesion of L-929 fibroblast and Chinese hamster ovary cell line. It was reported that the cell adhesion and proliferation of both cells was significantly improved on

CF_4-modified BC, while unremarkable increase of cells proliferation for O_2 or N_2-modified BC scaffolds was reported in comparison with pristine BC scaffold [80]. Kuzmenko et al. reported a universal method of protein bioconjugation on BC scaffolds in order to increase cell adhesion. The surface of BC scaffolds was modified with two proteins, fibronectin and collagen type I, through the bioconjugation applying 1-cyano-4-dimethylaminopyridinium (CDAP) tetrafluoroborate as the intermediate catalytic agent. Effective promotion of cell attachment by CDAP treatment to BC scaffolds was shown for human umbilical vein endothelial cells and the mouse mesenchymal stem cells [81]. Recently, the surface oxidized modification of BC with TEMPO-C6 or dialdehyde-C2, 3 was also reported to promote the adhesion and proliferation of cells [53,82].

3.2. Drug excipient and drug delivery

Possessing excellent compaction property, cellulose has a long history of application in the pharmaceutical industry, in particular as pharmaceutical excipients to condense drug-loaded matrices as suitable tablets for oral administration. Despite an extended history of use in tableting, there is still continuing research into the use of cellulose with new types of cellulose (viz. nanocellulose) in advanced drug-loaded systems whereby the rate of tablet disintegration as special excipients, or prolonged drug release as novel drug carriers. As a drug delivery excipient, Burt et al. investigated the capability of pure CNC to bind water soluble antibiotics (tetracycline and doxorubicin), and the potential of cationic-CNC to bind nonionized hydrophobic anticancer agents (docetaxel, paclitaxel, and etoposide) [83]. Besides direct use as excipient, CNC can also be used as co-stabilizer to improve the physicochemical and flow properties of polymeric excipients. Acrylic beads prepared via emulsion polymerization using CNC as co-stabilizer were proved to be a suitable excipient. The presence of CNC affected positively the size and size distribution of the bead excipient, which formed a stable structure together with low flow time and reduced cotangent of angle [84]. In another work, investigating spray-drying treatment on tablets, CNF exhibited a better ability to pack with lower powder porosity than commercial microcrystalline cellulose, which indicated novel spray-dried CNF excipient for tablet production [85]. With the same technique of spray-drying, BC was film-coated on tablets, and provided soft, flexible, and foldable nanocellulose films, which exhibited better mechanical properties in comparison with traditional Aquacoat ECD materials (polymeric materials composed of 30 wt% aqueous ethylcellulose dispersion) materials [86].

Common forms of nanocellulose-based drug carriers can be mainly divided into three aspects, viz. microspheres (or microparticles), hydrogels (or gels), and membranes (or films). Table 2 summarizes various drug carrier systems based on nanocellulose. It was reported that solid carriers formed from nanocellulose and different matrices spatially trapped drug molecules, and imparted the regulation of drug release. Lin et al. developed a pH-sensitive CNC/sodium alginate microsphere-based controlled release system for drug delivery. The presence of CNC in alginate-based microspheres showed more consistent swelling patterns, higher encapsulation efficiency, and promising sustained release profiles of the drug [87]. Regarding the application of nanocellulose in the fabrication of hydrogels, CNC was chemically grafted with cyclodextrin and participated in the architecture of hydrogels via in situ inclusion interactions. The drug release study revealed the performance of hydrogels as drug carriers for the in vitro release of doxorubicin and exhibited the behavior of prolonged drug release with special release kinetics and mechanisms, which were the "obstruction effect" and "locking effect" attributed to the good dispersion of the nanoparticles and the formation of a rigid network of CNC [91]. Kolakovic et al. reported the application of CNF films for long-lasting sustained drug delivery by a filtration processing. The drug release studies showed generally sustained drug release over periods of three months for model drugs. Interestingly, with the same CNF drug carriers, the release of indomethacin showed diffusion limited release, while itraconazole and beclomethasone showed almost zero-order release kinetics. The dependence of model drug used for release kinetics was attributed to the different drug solubilities in the dissolution medium, and the varied effects of drug binding to the CNF chains [100]. In another study, Valo et al. coupled a genetically engineered hydrophobin fusion protein with cellulose binding domains (CBD) and coated itraconazole drug nanoparticles for subsequent binding to CNF. Hydrophobin or hydrophobin-double CBD was selected to facilitate drug molecules binding to CNF matrix. The presence of CNF provided protection for drug nanoparticles and notably increased formulation storage stability during the formulation process and storage. It was reported that in the carrier system containing hydrophobin-coated CNF, drug nanoparticles around 100 nm could be stored for more than ten months [89]. Regarding the studies of BC in drug delivery, Huang et al. recently investigated the effects of BC membranes for the delivery of berberine hydrochloride and berberine sulfate in comparison with commercial tablet. It was reported that BC is a promising drug carrier to significantly extend the release duration of model drugs [104]. Müller et al. performed BC as a hydrogel carrier for bovine serum albumin as the model drug. It was shown that freeze-dried BC samples exhibited lower uptake of albumin than native, never-dried BC and that release of the model drug was a result of both diffusion- and swelling-controlled processes. Further studies using luciferase as the model protein indicated that the three-dimensional structure and activity of this protein can be preserved during the binding and release from BC hydrogels [94].

Unlike traditional trapping strategy, some researchers recently attempted to directly attach drug molecules on nanocellulose, which was performed using covalent coupling between modified nanocellulose and drug molecules. With a series of oxidation, reductive-amination, and esterification reactions in aqueous media, a novel CNC-based delivery system attached to the syringyl alcohol linker through a γ-aminobutyric acid spacer molecule can be produced, on which small model amine drugs (e.g., phenylpropanolamine) can be covalently connected [110]. Similarly,

Table 2
Drug carrier systems based on nanocellulose.

Carrier form	Material component		Model drug	Release time and medium	Mechanism model	Ref.
	Nanocellulose	Matrix				
Microsphere or bead	CNC	EA; MMA; BMA	Propranolol hydrochloride	12 h in pH 6.8 PBS	–	[84]
		Sodium alginate	Theophylline	16 h in pH 7.4, pH 6.8, pH 1.0 PBS	Ritger-Peppas equation	[87]
	CNF	–	Indomethacin; nadolol; atenolol; metoprolol tartrate; verapamil; ibuprofen	10–14 d in pH 7.4 PBS	Baker-Lonsdale mathematical model	[88]
		Hydrophobin	Itraconazole	90 min in pH 1.2 NaCl/HCl solution	–	[89]
Suspension	CNC	CTAB	Paclitaxel; docetaxel; DOX; TET; etoposide	1–4 d in PBS	–	[83]
		Chitosan oligosaccharide	Procaine hydrochloride	100 min in pH 8 NaCl solution	–	[90]
Hydrogel or gel	CNC	Cyclodextrin/ Pluronic	DOX	6.5 d in water	Ritger-Peppas equation	[91]
		Cyclodextrin/ Pluronic	Bovine serum albumin	20 h in pH 7.4 PBS	–	[92]
		Regenerated cellulose	Bovine serum albumin	48 h in simulated body fluid	Fickian diffusion law	[93]
	BC	–	Bovine serum albumin	48 h in pH 7.4 PBS	Ritger-Peppas equation	[94]
		–	Collagen; hyaluronan; growth factors	36–96 h in PBS	–	[95]
		Acrylic acid	Bovine serum albumin	8 h in simulated intestinal fluid	Ritger-Peppas equation	[96]
		Polyacrylamide	Theophylline	24 h in pH 7.4 PBS	Ritger-Peppas equation	[97]
Membrane or coating for tablet	CNF	–	Paracetamol	5–10 min in water	–	[85]
		–	Lysozyme	10 h in pure water or water/ ethanol solution	Fick's second law	[98]
		–	Caffeine	9 h in water	Higuchi equation	[99]
		–	Indomethacin	30 d in pH 5.0 phosphate buffer	Higuchi equation	[100]
			Itraconazole	90 d in pH 1.2 NaCl/HCl solution		
			Beclomethasone dipropionate	90 d in water		
	BC	–	Paracetamol	2 h in pH 5.8 PBS	–	[86]
		–	Lidocaine	7 h in pH 7.4 PBS	–	[101]
		–	Lidocaine; ibuprofen	8 h in pH 7.4 PBS	Fickian diffusion law	[102]
		–	Caffeine	15 h in pH 7.4 PBS	–	[103]
		–	Berberine hydrochloride, berberine sulfate	24 h in pH 2.1 HCl or H_2SO_4 solution; pH 6.8 PBS; pH 12.0 NaOH solution	Ritger-Peppas equation	[104]
		–	Glycerin	24 h in *in vivo* evaluation (skin)	–	[105]
		Poly(vinyl alcohol)	Vanillin	1 h in water	Ritger-Peppas equation; Fickian diffusion law	[106]
Nanofiber	CNC	Poly(lactic acid)	Columbia blue	48 h in water	Higuchi equation	[107]
		Hordein/zein	Riboflavin	24 d in pH 7.4 PBS	–	[108]
Aerogel	CNF	–	Beclomethasone dipropionate	700 min in pH 8.0 SDS solution	–	[109]

Abbreviations: BMA, butylmetacrylate; CTAB, cetyl trimethylammonium bromide; DOX, doxorubicin hydrochloride; EA, ethyl acrylate; MMA, methylmethacrylate; TET, tetracycline hydrochloride; PBS, phosphate-buffered solution; SDS, sodium dodecyl sulfate.

with the binding of bi-functional fusion protein on CNF and CNC, hydrophobic solid drug nanoparticles can be adsorbed by the packed protein film on nanocellulose and can improve the long-term stability of drugs under physiological condition [111].

The use of natural nanocellulose to deliver drugs is an attractive idea, but many issues are still under investigation, especially regarding the influence and regulation of drug release, interactions between drug molecules and nanocellulose [112], as well as possible reduction or destruction of drug activity and structure. Recently, it was reported that the surface charges on nanocellulose (TEMPO-oxidized negatively charged CNF) presented the adverse impact on the chemical stability of drug molecules (aspirin), which will accelerate the decomposition of drugs [113].

3.3. Immobilization and recognition of enzyme/protein

To overcome the problem of instability and rapid loss of catalytic activity during the operational and storage periods resulting from autolysis, unfolding, and aggregation of enzyme and protein, the immobilization of enzyme/protein on carriers has been considered as a powerful technique in diverse fields ranging from food technology to biomedical and biosensor engineering. An ideal carrier material for enzyme/protein immobilization should be biocompatible without compromising the protein structure and biological activity. Furthermore, this carrier material should be easily processed to enhance the enzyme/protein loading and activity as well as the stability in both operation and storage. As a nontoxic, noncarcinogenic, biocompatible, and in no way injurious in the biological environment material, nanocellulose meets the rigid medical requirements of suitable carrier for the immobilization of enzyme and protein. Another key point in enzyme/protein immobilization is the selection of the immobilization method, such as adsorption, entrapment, or covalent binding on carrier material. Available hydroxyl groups and possible negative charges (CNC and CNF) on the surface of nanocellulose provide the possibility of enzyme/protein immobilization on the basis of chemical conjunction and electrostatic adsorption. Table 3 summarizes recent studies on surface immobilization of enzyme or protein on nanocellulose on the basis of different strategies (chemical binding or physical adsorption). Covalent immobilization of enzyme/protein on nanocellulose can provide significantly high enzyme/protein loading and excellent stability, but always treated with complicated chemistry procedure. Physical approach is simple, cheap, and allows better preservation of the original structure of enzyme/protein, but with limited loading and efficiency of immobilization.

Table 3
Surface immobilization of enzyme or protein on nanocellulose.

Strategy	Type	Enzyme or protein model	Specific procedure	Ref.
Chemical conjunction	CNC	Lysozyme	• Esterification • Coupling with enzyme	[114]
		Peroxidase	• Surface activation • Coupling with protein	[115]
		Alcohol oxidase; CGTase	• Surface deposited gold nanoparticles • Coupling with enzyme	[116]
		Glucose oxidase	• Polyetherimide surface cationization • Deposited gold nanoparticles • Coupling with enzyme	[117]
		Tryptophan-based peptides	• Surface TEMPO-oxidation • Coupling with peptides	[118]
		Human neutrophil elastase	• Esterification with glycine • Coupling with elastase	[119]
		Papain enzyme	• Embedment of Fe_3O_4 and Au on the surface • Surface activation • Coupling with enzyme	[120]
		β-Casein	• Azide modification on CNC reducing ends • Acetylene modification on β-Casein • Click reaction for the coupling of protein	[121]
	CNF	Alkaline phosphatase; anti-hydrocortisone antibody	• Amine/epoxy/carboxylic acid modification • Coupling with protein	[122]
		Avidins	• Surface TEMPO-oxidation • Coupling with protein	[123]
Physical adsorption	CNC	Heptapeptide	• Adsorption and identification of peptide	[124]
		Diblock protein (Elastin-*co*-Cartilage oligomeric matrix)	• Copolymerization of diblock proteins • Adsorption of proteins	[125]
	CNF	Pancreatic serine protease trypsin	• Synthesis of nanocellulose-based hydrogel • Entrapment of protein	[126]
		Antihuman IgG antibody	• Oxidation and activation • Adsorption of protein	[127]
		Human immunoglobulin G (IgG)	• Surface grafting from poly(AMA-*co*-HEMA) • Adsorption of modified peptide (acetylated-HWRGWVA) • Coupling with protein	[128]
		Laccase	• Adsorption of enzyme	[129]
		Lipase	• Adsorption of enzyme	[130]
	BC	Hemoglobin; myoglobin; albumin; lysozyme	• Surface phosphorylation • Adsorption of protein	[131]
		Hemoglobin	• Surface quaternary ammonium • Adsorption of protein	[132]
		Glutamate decarboxylase	• Preactivation • Adsorption of enzyme	[133]
		Urease	• Surface modification with dye molecule • Adsorption of enzyme	[134]

Abbreviations: Poly(AMA-*co*-HEMA), poly(2-aminoethyl methacrylate hydrochloride-*co*-2-hydroxyethylmethacrylate).

Available hydroxyl groups and negative charges on the surface of CNC provide the possibility of enzyme/protein immobilization on the basis of chemical conjunction and electrostatic adsorption. Regarding the chemical strategy, some studies directly immobilized enzyme/protein on CNC with chemical grafting, such as immobilization of lysozyme on amino-glycine-CNC with carbodiimide-activation coupling reaction [114]; or peroxidase on CNC with the activation of cyanogen bromide treatment [115]. Another approach consists in functionalizing with smaller nanoparticles (generally gold nanoparticles, Au), and then realizing the immobilization of enzyme/protein on CNC with the aid of inorganic nanoparticles. Luong et al. investigated CNC/Au as a catalytic platform for enzyme immobilization, which exhibited significant biocatalytic activity and preservation of original activity. The recovered specific activities were about 70% and 95% for CGTase and alcohol oxidase enzymes, respectively [116]. More complicated carrier based on CNC/PEI/Au was developed to immobilize glucose oxidase enzyme [117]. Mahmoud et al. developed a special nanocomposite consisting of magnetite nanoparticles (Fe_3O_4) and Au nanoparticles embedded on CNC as a magnetic support for covalent conjugation of papain and facilitated the recovery of immobilized papain [120]. The conjugated material retained high enzyme activity and good stability and reusability. Based on the similar strategy of enzyme/protein immobilization, labeled DNA or enzyme was immobilized on CNC as a bioprobe, and used for the identification or recognition of target DNA sequence, enzyme molecules, or as the platform for immunoassays and diagnostics [135,136]. Edwards et al. reported a colorimetric approach for the detection of human neutrophil elastase (HNE) using peptide conjugated CNC [119]. Recently, the immobilizing effects of CNC and the diblock proteins bearing two different self-assembling domains [elastin (E) and the coiled-coil region of cartilage oligomeric matrix protein (C)] were investigated. It was reported that the protein CE with prevalent displaying of the E domain interacted more with CNC leading to a stronger network, while the protein EC, which is predominantly C-rich on its surface, did not interact as much with CNC. This study suggested that the surface characteristics of the protein polymers, due to folding and self-assembly, were important factors for the interactions with CNC, and therefore of significant influence on the overall immobilization efficiency [125]. With the purpose of identifying specific crystalline region of CNC for the immobilization of enzyme or protein, Guo et al. reported the phage display technology involving biopanning assays and enzyme-linked immunosorbent assay to investigate this binding property. A model of consensus peptide was efficiently immobilized on CNC, and the analysis indicated that peptide exhibited a bent structure when bound, allowing the Y5 amino acid to form a CH/π stacking interaction and H-bond with the glucose ring of cellulose [124].

Regarding chemical conjunction of enzyme or protein on CNF, some studies directly immobilized enzyme/protein macromolecules on CNF with grafting reactions, such as avidins binding on oxidized CNF [123], protein immobilization of alkaline phosphatase and anti-hydrocortisone antibody on amine, epoxy, and carboxylic acid modified CNF [122]. More studies reported the physical adsorption to immobilize enzyme or protein on CNF. In order to enhance the interactions of enzyme/protein immobilization on CNF, some studies attempted to modify the surface of CNF before the entrapment of enzyme/protein molecules, such as oxidation and activation pretreatments [127] and surface polymeric grafting [128].

The immobilization of enzyme/protein on BC is mainly achieved by physical interactions between BC and original enzyme/protein molecules, such as electrostatic adsorption of proteins on modified BC with surface phosphorylation or quaternary ammonium [131,132]. Recently, the properties and feasibility of BC membrane for the immobilization of glutamate decarboxylase was reported. With a pre-activation treatment followed by protein adsorption, immobilized glutamate decarboxylase on BC membrane exhibited good retention of protein activity (89.17%), least leakage, and high stability (5% loss), which was associated to the porosity of the carrier material [133].

3.4. Substitutes/medical biomaterials

Promising mechanical properties and good biocompatibility of nanocellulose promote its research and development as substitute/medical biomaterial, such as the replacement of blood vessel (vascular graft), soft tissue, and nucleus pulposus. The studies of nanocellulose as blood vessel replacement are most attracting and fruitful, as reported from the effects in various animal experiments before clinical research. Regarding the studies on nanocellulose as soft tissue and nucleus pulposus, most reports are still in the fundamental stage, and mainly focus on the comparison of different properties between nanocellulose-based materials and real organs.

3.4.1. Blood vessel replacement

One of the most common treatments to cardiovascular disease is the coronary bypass graft surgery, which is performed to supply blood to the heart tissue with a suitable blood vessel replacement. Possessing good mechanical strength (a burst pressure of up to 880 mmHg) and blood biocompatibility, it is possible to develop nanocellulose (especially for BC) as material for artificial tubes used as potential replacement of small (<4 mm) or large (>6 mm) size vascular grafts. The team of Dieter Klemm (University Jena and Polymer Jena, Germany) was the first research organization to investigate and apply artificial vascular substitute obtained with biomaterials from BC. They have discussed the application of BC as blood vessel replacement in some publications [137–139], and especially described a clinical product named BActerial SYnthesized Cellulose (BASYC®) with high mechanical strength in wet state, enormous water retention property, and low roughness of inner tube surface. It is reported that BASYC® from BC has been successfully used as the artificial blood vessel in rats and pigs for microsurgery [140,141]. In comparison with conventional synthetic vascular graft materials, e.g. polyester (Dacron) and ePTFE, biosynthetic BC tubes can be suitable for small diameter (<4 mm) vascular conduits, and restrain the phenomena of thrombus induction and stenosis. BASYC, BActerial SYnthesized Cellulose (BC tubes) with different

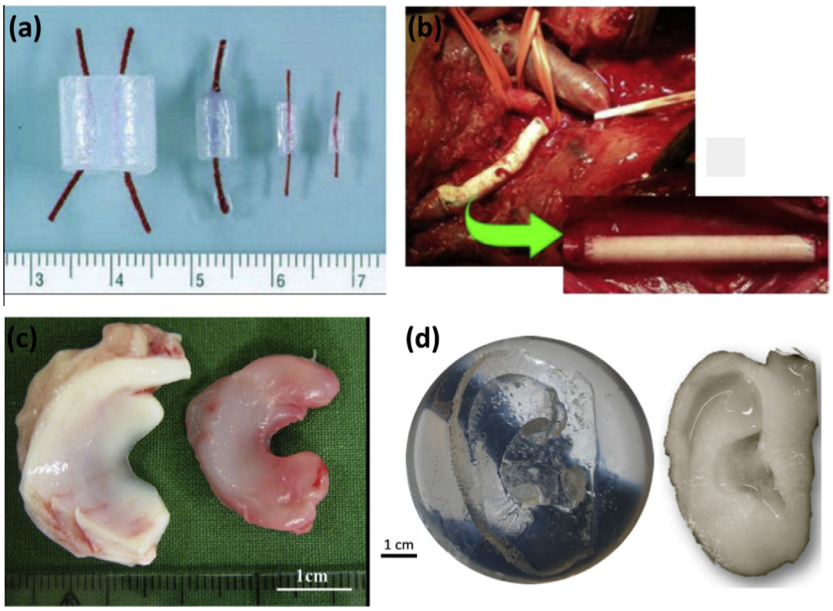

Fig. 4. Examples of substitutes from nanocellulose. (a) BASYC, BActerial SYnthesized Cellulose (BC tubes) with different dimensions (adapted with permission from [145]). (b) Vascular prostheses made of CNF-polyurethane placed between the brachiocephalic trunk and the right common carotid artery in male patient (adapted with permission from [146]). (c) Comparison between pig meniscus (left) and BC hydrogel (right) (adapted with permission from [147]). (d) Negative silicone mold used to guide the bacteria during the bacterial culture to reproduce the large-scale features of the outer ear (left); and 3D BC implant prototype (1% effective cellulose content) produced in the shape of the whole outer ear according to the 3T MRI scanning technique (right) (adapted with permission from [148]).

dimensions are shown in Fig. 4(a). Recently, various properties and biology evaluation of BC tubes as blood vessel replacement have been investigated, involving the issues of BC biomaterial-induced coagulation [142], cell attachment, proliferation, viability and invasion [143], hemodynamic analysis and microcirculatory evaluation [144], etc.

Different from BC biosynthetic procedure, it is impossible to directly fabricate the tubes from CNC and CNF. Therefore, the development of CNC or CNF-based blood vessel replacement commonly includes the use of a matrix material. Recently, Brown et al. reported the synthesis of CNC/fibrin biocomposites for the potential application of small-diameter replacement vascular graft. CNC was covalently grafted on fibrin matrix, and provided nanoreinforcement in terms of strength and elasticity to the composites [149]. However, this material has not been tested *in vivo*. Novel biomaterials from polyurethane reinforced with CNF have been reported to be potentially used as vascular replacement. The presence of CNF in polyurethane improved the elastic properties of the material, coupled with low thrombogenicity and exceptional physical and mechanical properties. CNF/polyurethane biomaterials, with a wall thickness of 0.7–1.0 mm, were applied as vascular prostheses between the brachiocephalic trunk and the right common carotid artery in a 26-year-old male patient with multiple endocrine neoplasia 2B (Fig. 4(b)). It is a pity that no further effect of this CNF/polyurethane biomaterial in clinical study was reported.

Based on decades of research, it is successful to produce nanocellulose-based materials for blood vessel replacement under proper control of the biotechnological formation. However, detailed *in vivo* characterization of the performance of these nanocellulose implants (in large animal and even human body studies) are still required, addressing in particular their long-term stability and suitability to replace small-caliber blood vessels without significant thrombogenicity, study of compliance between the graft and the surrounding native vessels, together with the postoperative complications

3.4.2. Soft tissue–ligament, meniscus, and cartilage replacements

The design and fabrication of materials suitable for soft tissue replacement are important aspects of the biomedical application. The demand for biomaterials to be used for soft tissue replacement should not only provide similar mechanical properties as the tissue it replaces, but also improve lifespan, biocompatibility, durability, and low degree of calcification. Using a double-network method, BC/polyacrylamide (PAAm) gels can be synthesized by combining BC gel as the first network, and PAAm as the second network in the presence of a crosslinker. The BC/PAAm gels presented high elongation and high tensile fracture stress (40 ± 10 MPa), which was similar to the tensile fracture stress of ligament (38 ± 10 MPa), and could be potentially used as ligament replacement [150]. Mathew et al. also reported the preparation of CNF and CNF/collagen composites for potential ligament. Both composites exhibited mechanical properties and stress relaxation behavior comparable to those of natural ligaments and tendons. Further *in vitro* biocompatibility study on these composites showed a positive response concerning adhesion/proliferation and differentiation for both human ligament and endothelial cells [151,152].

Gatenholm et al. compared the mechanical properties of BC gel with traditional collagen meniscal implant material and real pig meniscus. It was reported that the Young's modulus of BC gel is similar to the one of pig meniscus, and five times higher than the one of collagen material. The results of promising cell migration and controlled meniscus shape (as shown in Fig. 4(c)) indicated that BC can be an attractive material as meniscus implant [147]. Another study investigated the friction and wear behaviors of BC pellicles against bovine articular cartilage. The tribological assessment of the sliding pairs for BC was performed using reciprocating pin-on-flat tests. Due to the wear mechanism involving high plastic deformation combined with the formation of tribological rolls at the contact interface, BC biomaterials possessing low friction coefficient values (about 0.05) and preservation of the mating surfaces can be obtained. This BC biomaterial was reported to be a potential replacement of artificial cartilage for articular joints [153]. Recently, based on the 3T MRI scanning technique, an ear-shaped BC prototype material was produced from a negative ear mold, as shown in Fig. 4(d). Meanwhile, it was reported that the mechanical properties of BC biomaterials can be regulated by the effective cellulose contents. This study proved that BC is a promising material to reach mechanical properties of ear cartilage replacement, and can be produced in patient-specific ear shapes [148].

3.4.3. Nucleus pulposus replacement

Nucleus pulposus is a gelatinous core inside two vertebral bodies for intervertebral disks, which is important to provide flexibility and dissipate the stresses acting on the spine. It is reported that about 80% of the world population suffers from back pain, and in 75% of cases this is a direct consequence of degenerative processes of the disc, in particular nucleus pulposus degeneration. In recent studies, a biocomposite hydrogel with carboxymethylated CNF was prepared by UV polymerization of *N*-vinyl-2-pyrrolidone for the replacement of native human nucleus pulposus. The biocomposite hydrogel containing 0.4% v/v of carboxymethylated CNF with DS of 0.17 presented a close behavior to native nucleus pulposus, such as low strain values after cyclic compression tests, and similar relaxation properties [154]. Further study demonstrated that this biocomposite hydrogel can act as a potential nucleus pulposus implant attributed to its adequate swelling ratio and improved mechanical properties, which may be beneficial to restore the annulus fibrosus loading and the height of the intervertebral discs [155].

3.5. Advanced nanomaterials for tissue repair, regeneration and healing

Tissue repair and regeneration is the process of renewal, restoration, and growth that makes the function of diseased and damaged cells, organs, and tissues resilient to natural fluctuations. From bacteria to humans, all species have specific ability of tissue repair and regeneration. Different from the effects of substitute implants, the behavior of tissue repair and regeneration for organism inherently originates from the individual self. Although no property of tissue regeneration or repair for nanocellulose itself, it can provide a nontoxic and biocompatible platform to cover growth factors or cells, which will activate and accelerate the process of tissue repair and regeneration. Most studied applications of nanocellulose-based biomaterials for tissue repair, regeneration, and healing are skin tissue repair (wound dressings) and bone tissue regeneration and healing.

3.5.1. Skin tissue repair and wound healing

Regarding skin repair materials (also called wound dressings), an important characteristic is their ability to absorb exudate during the dressing process, and its removal from a wound surface after recovery. The drawbacks of traditional skin tissue repair materials, e.g. gauze, are their strong permeability, which will cause the tight adhesion of repair materials on the desiccated wound surface and thus induce new trauma on removal [156]. Considering its significant biological properties, interest on nanocellulose (especially BC) for novel wound care has steadily increased. BC skin tissue repair biomaterials can be fabricated by a multilayer fermentation method, which showed low cytotoxicity and good proliferation of human adipose derived stem cells. According to the animal experiments and histological examinations, more rapidly fresh tissue regeneration and significant capillary formation in the wound area with BC-based biomaterials were reported compared with commercial dressings [157,158]. In another study, the effects of BC as wound dressing material were evaluated on animal experiments (male 6-week-old Sprague-Dawley rats), which proved that the presence of BC can promote wound healing by accelerating contractions through the accumulation of extracellular matrix [159]. Some studies also attempted to combine nanocellulose with various natural matrices in order to develop enhanced biocomposites for potential skin tissue repair materials, such as collagen [160], gelatin [161], alginate [162], chitosan [163], cotton gauze [164], poly(ethylene glycol) [165], and poly(vinyl alcohol) [166].

BC-based biomaterials have been reported to be applied in clinical practice. Non-healing lower extremity ulcers were treated with a BC wound dressing. The time required for 75% reduction in wound size was compared for 11 chronic wounds with and without the presence of BC. The mean period of wound healing without the addition of BC was 315 days (95% confidence interval (CI): 239–392 days), while with the incorporation of BC to these chronic wounds, the mean time of wound healing reduced to 81 days (95% CI: 50–111 days). In this case, the use of BC wound dressing was reported to significantly shorten the time for the tissue repair of non-healing lower extremity ulcers compared with standard care [167]. In the studies of clinical effects of BC for skin tissue repair, clinical trials were conducted on 34 patients suffering from severe thermal burns covering 9–18% of the total body surface area, in which 22 of the patients received BC as testing group. It was reported that the adherence of BC membrane to the wound surface was excellent in avoiding dead spaces, which indicated that the application of BC dressing in the treatment of partial thickness burns to promote a favorable environment for fast wound cleansing and rapid healing [168]. Similar conclusions on BC improving skin tissue

repair in clinical research were also reported in recent publications [169,170].

On the basis of fundamental researches on the development of BC-based skin repair materials, some companies have launched several commercial products in wound healing system. BioFill Produtos Bioetecnologicos (Curitiba, PR Brazil) developed a series of products based on BC, including Biofill® and Bioprocess® (used in the therapy of burns, ulcers as temporary artificial skin), and Gengiflex® (applied in treatment of periodontal diseases). Another company, Xylos Corporation in the US, has developed several medical devices using BC since 1996. The XCell® family offered by Xylos Corporation has been marketed in the US since 2003. Unlike BC dressings manufactured by Biofill®, the Xcell® product is claimed to have a dual-function of both hydration and absorption to maintain the ideal moisture balance [171].

3.5.2. Bone tissue regeneration and healing

Developing effective bone regeneration therapy is a long-term attracting clinical topic. Bone loss caused by trauma, neoplasia, reconstructive surgery, congenital defects, or periodontal disease is a major health problem worldwide. As mentioned in the Section 3.1, nanocellulose and its biocomposites have been proved to be the promising scaffolds for the culture of various cells, including osteoblast and chondroblast, which indicates that nanocellulose-based materials have the potential for bone tissue regeneration and healing. However, studies on nanocellulose for bone tissue regeneration and healing applications are still at the fundamental stage, and only few publications report the practical effects on animal experiments. A membrane composed of BC and hydroxyapatite was developed as biomaterial for potential bone regeneration, which showed the promotion of growth of osteoblast cells, high level of alkaline phosphatase activity, and greater bone nodule formation. The better osteoblasts adhesion, proliferate and mineralization from BC/hydroxyapatite biomaterials were expected to facilitate quick regeneration of bone tissue [172]. Saska et al. further evaluated the biological properties and practical effects of BC/hydroxyapatite membranes for bone regeneration with *in vivo* animal experiments. The biomaterials were embedded to improve noncritical bone defects in rat tibiae at 1, 4, and 16 weeks. Low crystallinity hydroxyapatite crystals presented a Ca/P molar ratio of 1.5, similar to physiological bone. The BC/hydroxyapatite membranes were proved to accelerate new bone formation at the defect sites for bone regeneration in rat tibiae according to *in vivo* tests for 4 weeks [173]. Recently, goat bone apatite was reported to be introduced in BC for the fabrication of novel bone repair biomaterials, which can stimulate bone cell proliferation and promote the cell differentiation. However, no *in vivo* experiment was reported in this study [174].

3.5.3. Other tissue repair

Recently, the fabrication of a nanofibrillar patch by using BC and its application as a wound-healing platform for traumatic tympanic membrane (ear drum) perforation was reported. The nanostructured surface, biocompatibility, transparency, and appropriate mechanical properties were expected to meet the requirements of an ideal wound-healing platform for tympanic membrane perforation. The tympanic membrane cells were found to well adhere and proliferate on the BC nanofibrillar patch, and *in vitro* the growth and migration of cells were promoted under the guidance of BC patch. Specific effects of BC patch materials on the regeneration and healing of tympanic membrane tissues were investigated through *in vivo* animal study (12 weeks Sprague-Dawley rats). It was reported that the presence of BC patch materials significantly increased the tympanic membrane healing rate as well as recovered the function of tympanic membrane better than spontaneous healing [175].

BC was reported to be developed as biomaterial for the reconstruction of damaged peripheral nerves via cellulosic guidance channels. *In vivo* experiments were conducted on the femoral nerve of Wistar rats for three months. Results evaluation from histological analysis and postoperative observation of motor recovery showed that BC neurotubes can effectively prevent the formation of neuromas, while allowing the accumulation of neurotrophic factors inside, and facilitating the process of nerve regeneration [176].

3.6. Antimicrobial nanomaterials

Wound infection caused by high bacterial levels, especially the burn wounds, traumatic injuries, and surgical procedures, is a significant reason for delayed or prolonged wound healing. Adherence and survival of pathogenic bacteria on the surface of wounds leading to concomitant transmission to new hosts significantly contribute to the proliferation of pathogens, which considerably increases the threat to human health. With increasing awareness of infectious diseases and antibiotic resistance, many studies were dedicated to the development of effective surface disinfection and alternative materials bearing antimicrobial and other bioactive characteristics, viz. antimicrobial wound dressing. Nanocellulose can provide a porous network structure in the architecture of biomaterials, which is beneficial for potential transfer of antibiotics or other medicines into the wound, meanwhile serving as an efficient physical barrier against any external infection [177]. It is also reported that antimicrobial nanomaterials from nanocellulose (carbohydrate nature) commonly exhibited compatibility with biological tissue as well as significant bioavailability and biodegradability [178,179]. However, because of the fact that nanocellulose itself has no antimicrobial activity and cannot prevent wound infection, nanocellulose-based antimicrobial biomaterials are generally achieved by the conjunction of antimicrobial agents and nanocellulose using physical or chemical approaches. According to different types of antimicrobial agents, nanocellulose-based antimicrobial biomaterials can be divided in two parts, including nanomaterials incorporated with inorganic antimicrobial agents (mainly involving silver particles (Ag) and its derivatives), and organic antimicrobial agents (e.g. lysozyme).

It is reported that among the different antimicrobial agents, silver has been most extensively studied and used since ancient times to fight infections and prevent spoilage. The silver nanoparticles with effective antibacterial,

antifungal and antiviral properties are proved as a promising antibacterial agent [180]. Chemical reduction ($AgNO_3$/reducing agent) and simple impregnation are common approach to introduce a silver antimicrobial agent into nanocellulose-based materials. The antimicrobial efficacy of Ag nanoparticle in nanocellulose-based biomaterials depends on the size and shape of synthesized nanoparticles. It was reported that CNC nanohybrid materials containing dendritic nanostructured Ag showed better antibacterial activity than that of sphere nanostructured Ag [181]. Recent studies attempted to incorporate both Ag nanoparticles and CNC in polymeric matrices to offer the synergy effects of antibacterial and mechanical reinforcement, such as poly(lactic acid)/CNC/Ag [182,183], and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)/CNC/Ag materials [184] and waterborne polyurethane/CNC/Ag [185]. Regarding the antibacterial nanomaterials from CNF and Ag nanoparticles, it was reported that composites composed of CNF and Ag nanoparticles can be fabricated by an electrostatic assembly approach via polyelectrolytes as macromolecular linkers between CNF and Ag nanoparticles [186]. Fluorescent silver nanoclusters were dipped into CNF materials with the mediation by poly(methacrylic acid). The presence of fluorescent silver nanoclusters provided both fluorescence and antibacterial activities for the composites [187]. The studies of Ag nanoparticle or its derivatives (AgCl, silver sulfadiazine) introduced in BC to develop BC/Ag antibacterial nanomaterials were most intensively studied, which exhibited a high antimicrobial activity [188–193,179] and good biocompatibility. Besides Ag nanoparticles, recent studies reported that zinc-oxide nanoparticles (ZnO) on CNC [194], CNF [195] or BC [196] materials also showed some antibacterial effects.

With the aim of avoiding toxicity and unsustainable effect of inorganic Ag nanoparticles, some novel organic antibacterial agents, were incorporated in nanocellulose to develop novel antibacterial materials. The reported organic antibacterial agents used in nanocellulose-based materials include CNC: porphyrin; CNF: octadecyldimethyl (3-trimethoxysilylpropyl) ammonium chloride [197], allicin and lysozyme [198], chitosan–benzalkonium chloride, chitosan–methylisothiazolinone; and BC: gentamicin, ε-polylysine [199], benzalkonium chloride [200], sorbic acid [201,202]. CNC was reported to be covalently grafted on cationic porphyrin groups, which showed excellent efficiency of photodynamic inactivation towards bacteria. This strategy was expected as the development of potential photobactericidal nanomaterials [203,204]. Liu et al. reported the preparation of sodium alginate/CNF antibacterial composites with the addition of chitosan–benzalkonium chloride or chitosan–methylisothiazolinone as antibacterial agents. Both antibacterial agents were nanospherical shape (30 nm and 10 nm) and adsorbed on the surface of CNF during several min, under the driving forces of hydrogen bonds and electrostatic interactions. Furthermore, these composites were reported to display promising mechanical strength and excellent antibacterial activity against *Staphylococcus aureus* [205,206]. In order to enhance the antibacterial activity of BC, gentamicin-activated BC membranes were prepared by chemically grafting RGDC peptides (R: arginine; G: glycine; D: aspartic acid; C: cysteine) with the crosslinking followed by covalent attachment of gentamicin onto the surface of the BC membrane network. It was reported that these gentamicin–RGDC-grafted BC membranes were bactericidal against *Streptococcus mutans* but nontoxic to human dermal fibroblasts, which showed potential application in wound healing or drug delivery systems [207].

Recently, some researchers studied the antibacterial property of nanocellulose with surface derivatization, which means the development of nanocellulose antibacterial materials without the use of antibacterial agents. Via a nucleophilic displacement reaction starting from cellulose-*p*-toluenesulfonic acid ester, the surface of CNF can be amino-functionalized. Interestingly, it was reported that electrospun PVA nanofibers containing this amino-modified CNF (with 6-deoxy-6-trisaminoethyl-amino agent at a degree of substitution of 0.67) exhibited a high antimicrobial activity against *S. aureus* and *Klebsiella pneumonia* [208]. However, it was a pity that the antimicrobial mechanism from this amino-modified CNF was not investigated. Similar study was also reported on chemical grafting of aminoalkyl groups onto the surface of BC nanofibrillar network to provide its antimicrobial activity. In this study, the chemical structure of amino-modified cellulose was compared similarly with chitosan, which was regarded as the origin and mimicking of antimicrobial property for modified BC [209]. Butchosa et al. reported the use of partially deacetylated chitin nanocrystals in BC materials to develop all-polysaccharide antimicrobial composites. It was reported that this "green" composite with all natural components showed strong antibacterial activity with 99 ± 1% inhibition of bacterial growth [210]. Recently, Ul-Islam et al. reported that BC composites incorporated into small concentrations of Cu-montmorillonite exhibited some antimicrobial activity, but without any mechanism investigation [211].

Most reported materials with nanocellulose with or without antimicrobial agents in the forms of suspension, composite, porous membrane/film, and electrospun nanofiber, all present promising antimicrobial effects against, e.g. Gram-positive bacterium (*S. aureus*), and Gram-negative bacterium (*Escherichia coli*). However, many interesting properties and pivotal issues on nanocellulose-based antimicrobial biomaterials are still unknown, especially regarding the reasonable balance between the improvement of antimicrobial activity, duration of antimicrobial effect, and control of normal human cell damage.

3.7. Other biomedical applications

Besides the traditional biomedical applications discussed before, nanocellulose has been attempted to be used in some new fields with special functions. A highly porous CNF/polypyrrole composite was developed as an electrochemically controlled solid phase extraction biomaterial for the capture of DNA oligomers. This biocomposite possessed a total anion exchange capacity of about 1.1 mol/kg, and was reported to extract and release the negatively charged fluorophore-tagged DNA oligomers through the galvanostatic oxidation and reduction of conformal polypyrrole layer (30–50 nm) on CNF substrate. Resulting from the high surface area of porous structure, the ion exchange

capacity of CNF/polypyrrole composite bore two orders of magnitude higher than traditional ion exchange material, and showed faster and better control of the polypyrrole charge for the capture of DNA oligomers [212]. In another study, this CNF/polypyrrole composite prepared with the same strategy and source of CNF, was applied as the hemodialysis membrane to purify blood. It was reported that this biomaterial exhibited an effective removal of small uremic toxins in blood and an improvement in thrombogenic properties with the coating of heparin, which were attributed to superior ion exchange capability and large surface area of the membrane. It should be also noted that due to the introduction of natural CNF, the hemocompatibility of this composite biomaterial was much better than commercial synthetic membranes (such as polysulfone) [34,213].

Tabuchi et al. reported the ability of BC medium to separate DNA fragments due to a double-mesh concept combined with a stereo effect from BC-intrinsic three-dimensional micrometer- to a nanometer-network structure. It was shown that a solution of 0.49% hydroxypropylmethyl cellulose polymer containing 0.3% BC fragments allowed excellent separation for a wide range of DNA sizes (10 bp–15 kbp) as well as a high resolution of single-nucleotide polymorphisms even though the viscosity of BC medium was less than 5 cP [214].

BC was also reported as an innovative material for dental root canal treatment. In comparison with conventional paper point materials, BC showed greater compatibility and biological characteristics for dental root canal treatment. The absorption rate of BC-based biomaterials was about 10-fold greater than that of paper point materials, and BC-based biomaterials can preserve better tensile strength under wet condition meeting the requirement of high-expansion of dental root canal biomaterials. In addition, it was reported that when used for dental root canal treatment in animal experiments, BC-based biomaterials showed maintenance of physical integrity, and only a small foreign body reaction [215].

4. Functional modification of nanocellulose for potential biomedical application

Diverse biomedical applications of nanocellulose discussed in Section 3 are exciting, but modification of

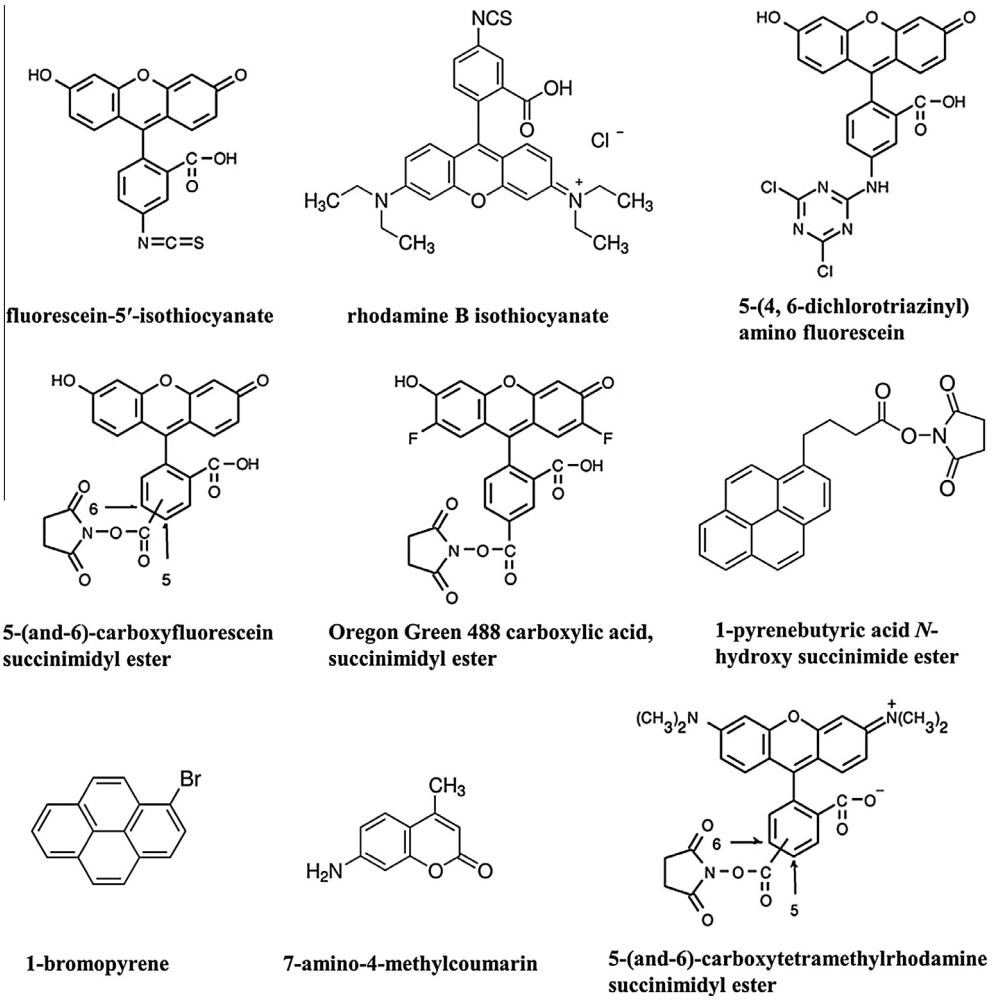

Fig. 5. Chemical structure of some fluorescent labeling molecules grafted on CNC.

nanocellulose before the development of practical materials is also important, which will determine its potential biological applications.

By the means of introducing fluorescent molecules on the surface, nanocellulose can be converted to functional nanoparticles with fluorescent labeling ability. It is expected that fluorescent modification on nanocellulose enables the potential use in biomedical fields, such as optical bioimaging, biosensor, and photodynamic therapy. On the other hand, characterized by various fluorescence techniques, fluorescent labeled nanocellulose is easier to be traced and evaluated for toxicity and bioactivity in materials. Since the first report of fluorescent labeling on CNC with fluorescein-5′-isothiocyanate (FITC) molecule [216], more and more studies focus on this topic. To date, diverse fluorescent molecules have been attempted to covalently attach on the surface of CNC, including FITC, Rhodamine B isothiocyanate [62], pyrene dyes [217], terpyridine and its derivatives [218], 1-pyrenebu-tyric acid *N*-hydroxy succinimide ester [219], 5-(and-6)-carboxyfluorescein succinimidyl ester, 5-(and-6)-carboxytetramethylrhod-amine succinimidyl ester, Oregon Green 488 carboxylic acid, succinimidyl ester [220], PEI-chlorin p6 derivatives [221], 5-(4, 6-dichlorotriazinyl) aminofluorescein [222], and 7-amino-4-methylcoumarin [223]. Fig. 5 shows the chemical structure of some fluorescent molecules that have been grafted on CNC. It is sure that there is still a long way for practical application of fluorescent CNC in biology, but it is also undoubted that the fluorescently modified CNC is so attracting that the breakthrough in this topic may bring the revolution of biomedical materials.

Surface grafting of amino acid molecules can offer biologically active building blocks on nanocellulose, which may contribute to the potential of nanocellulose to be used as a nanocarrier for DNA delivery. Chemical conjunction between amino acid and CNC or CNF can be achieved with two strategies: (1) esterification reaction between Fmoc-amino acid and CNC, and removal of Fmoc-protecting group [224]; (2) activation of oxidized CNF to form a stable active ester, and grafting of amino acid with the formation of novel amide bond [225]. Recently, using the molecular recognition ability of DNA oligomeric base pairs, duplexing complementary DNA oligonucleotides have been grafted onto CNC to produce DNA-based biocompatible nanomaterials, which may be used as special biomaterials for enzyme/protein immobilization [226]. Ferrocene-decorated CNC can be prepared by grafting ethynylferrocene onto azide functionalized CNC using azide–alkyne cycloaddition reaction, which can be assembled in three-dimensional structures for potential application in biosensors and bioelectrochemical assemblies [227].

5. Conclusions and remarks

The aim of this article is to demonstrate the current state of research and future development of nanocellulose in the application of biomedicine through the discussion of selected examples. Undoubtedly, nanocellulose has great potential for the breakthrough of a novel generation of biomedical materials. Reported studies on nanocellulose have led to significant advancement with the promise of even greater advances likely to come in the future. Overall, creating controlled properties, reliable and reproducible production techniques for biocompatible nanocellulose (not only for BC) will be essential and beneficial to pave the way for greater acceptance of nanocellulose as a commercially available material in biomedical applications. Further comparison and investigation on different effects of the three types of nanocellulose (CNC, CNF and BC) will determine their respective applications in biomedical materials.

Specifically, regarding cellular bioscaffold, the mechanisms for cells and nanocellulose interaction remain enigmatical and require intensive *in vivo* study. Furthermore, on the basis of mechanism analysis, it is possible for future study to regulate the interactions between cells and nanocellulose through controlling the macro- and microstructure of nanocellulose. Different pharmaceutical molecules, together with growth factors, or antigenic factors will be combinatorially organized in nanocellulose-based drug carriers, and used for synergically medical therapy purposes. The studies on the development of tissue substitutes and repair biomaterials have made positive progress (especially with BC), which promotes the launch of several commercial products and practical usage in clinic. On the one hand, novel nanocellulose-based tissue substitutes and repair biomaterials will be more versatile with the possible incorporation of biocompatible factors or functional factors (such as anticoagulant factors). On the other hand, covalent attachment of biologically active ligand molecules to the nanocellulose framework can enhance and alter its characteristics for specific applications, which may improve interactions between materials and human tissues.

From both scientific and economic viewpoints, nanocellulose, the resource and gift provided by Nature, is on the threshold of a breakthrough driven by recent extraordinary activities in the field of biomedical applications.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eurpolymj.2014.07.025.

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Ning Lin is currently conducting post-doctoral research at Université Joseph Fourier and Grenoble Institute of Technology in France. He received his PhD in 2014 at the International School of Paper, Print Media and Biomaterials (Pagora) in Grenoble Institute of Technology under the supervision of Prof. Alain Dufresne. His research interests involve surface modification, design, development and application of nanomaterials based on nanopolysaccharide.

Alain Dufresne received his PhD in 1991 from the Department of Electronic at the Toulouse National Institute of Applied Science. He is professor at the International School of Paper, Print Media and Biomaterials (Pagora) in Grenoble Institute of Technology. His main research interests concern the processing and characterization of polymer nanocomposites reinforced with nanoparticles extracted from renewable resources.