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REVIEW

# Recent advances in nanocellulose for biomedical applications

**Mehdi Jorfi,[1]* E. Johan Foster[1,2]**

[1]Adolphe Merkle Institute, University of Fribourg, Chemin des Verdiers 4, Fribourg CH-1700, Switzerland

[2]Department of Materials Science and Engineering, Virginia Tech, 445 Old Turner Street, 213 Holden Hall, Blacksburg, Virginia 24061

*Present address: Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139

Correspondence to: M. Jorfi (E-mail: jorfi@mit.edu) and E. J. Foster (E-mail: johanf@vt.edu)

**ABSTRACT:** Nanocellulose materials have undergone rapid development in recent years as promising biomedical materials because of their excellent physical and biological properties, in particular their biocompatibility, biodegradability, and low cytotoxicity. Recently, a significant amount of research has been directed toward the fabrication of advanced cellulose nanofibers with different morphologies and functional properties. These nanocellulose fibers are widely applied in medical implants, tissue engineering, drug delivery, wound-healing, cardiovascular applications, and other medical applications. In this review, we reflect on recent advancements in the design and fabrication of advanced nanocellulose-based biomaterials (cellulose nanocrystals, bacterial nanocellulose, and cellulose nanofibrils) that are promising for biomedical applications and discuss material requirements for each application, along with the challenges that the materials might face. Finally, we give an overview on future directions of nanocellulose-based materials in the biomedical field. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41719.

**KEYWORDS:** biomaterials; biomedical applications; cellulose; composites; functionalization of polymers

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## INTRODUCTION

In recent years, intense research has focused on the use of natural polymers in a variety of biomedical materials and devices, including wound dressings, medical implants, drug delivery, vascular grafts, and scaffolds for tissue engineering.[1–3] These natural polymers are present in a wide variety of natural organisms with properties tailored to meet the specific needs of living organisms; thereby, they carry interesting properties of the respective tissues and similar ones to the extracellular matrix. These natural polymers, including alginate, chitosan, gelatin, collagen, elastin, starch, and cellulose, have received increasing attention for various uses in biomedical and pharmaceutical applications. Cellulose, in particular, has been the subject of intensive research because of its sustainability, biodegradability, and biosafety, and has been used extensively in recent years in the biomedical field.

Cellulose is the most abundant natural polymer on Earth, with a bioproduction estimated to be over $7.5 \times 10^{10}$ metric tons annually.[4] Cellulose is widely distributed over a variety of sources, including marine animals (e.g., tunicates), plants (e.g., wood, cotton, or wheat straw), and bacterial sources, such as algae (e.g., *Valonia*), fungi, and even amoeba (protozoa). Regardless of its source, cellulose is a fibrous, tough, linear, syndiotactic homopolymer composed of D-anhydroglucopyranose units, which are connected by $\beta$-(1→4)-glycosidic bonds. On the basis of the source of the cellulose and the chemical treatment, the resulting fibers can vary in several properties, including morphology, aspect ratio, surface chemistry, crystal structure, and degree of crystallinity.[4,5] Because of their strongly interacting hydroxyl groups, cellulose materials have a strong tendency to self-associate and form an extended network via both intramolecular and intermolecular hydrogen bonds.[4,6–9] These intramolecular and intermolecular interactions between cellulose chains and/or solvents have been discussed in detail elsewhere.[10–14] To date, many researchers have investigated the design, fabrication, and processing of cellulose materials for potential use in medicine. Widespread interest in nanocellulose-based biomedical materials has been centered on their low cost, biodegradability, biocompatibility, outstanding mechanical properties, availability, sustainability, and low cytotoxicity. Among the many types of nanocellulose materials, bacterial nanocellulose (BNC; also referred to as microbial cellulose or MC) has been a widely exploited cellulose in recent years for a wide range of biomedical applications, and it has already been used successfully in wound-healing and tissue engineering applications.[15–17] BNC-based dressing materials, such as XCell,

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**Mehdi Jorfi** is a postdoctoral fellow in the Department of Chemical Engineering at the Massachusetts Institute of Technology in Cambridge, Massachusetts. He received his Ph.D. in biomaterials science and engineering at the Adolphe Merkle Institute at the University of Fribourg (Switzerland) in 2014 under the supervision of Christoph Weder and E. Johan Foster. His Ph.D. work was focused on the development and investigation of polymeric biomaterials for a broad range of biomedical applications. More specifically, he designed various smart biomaterials for neural interfacing applications, including polymeric biomaterials capable of delivering various drug molecules directly into the brain. His current research interests include bio-inspired materials, dynamic stem cell microenvironments, organs-on-chips, drug delivery, stem cell engineering, and regenerative medicine.

**E. Johan Foster** is an associate professor at Virginia Tech in the Department of Material Science and Engineering. He is a member of the Macromolecules and Interfaces Institute at Virginia Tech and the Virginia Tech Center for Sustainable Nanotechnology. He was educated at Simon Fraser University (Canada) and was a postdoctoral fellow with Prof. E. W. (Bert) Meijer at Technical University Eindhoven (The Netherlands). Previously, he held the position of maître assistant at the Adolphe Merkle Institute in Fribourg, Switzerland. He focuses on advanced functional and supramolecular bionanomaterials: the design, synthesis, and engineering of bio-inspired, biosourced functional polymers, supramolecular materials, and NCs; stimuli-responsive materials; and biomedical materials. Often, his materials combine covalent and noncovalent interactions to create structured smart materials.

Bioprocess, and Biofill, are already commercially on the market for topical applications in wound healing; this proves that they could become high-value products in the medicine field.[15]

In the last decade, numerous nanocellulose-based materials have been created for a variety of biomedical applications. There has been a tremendous increase in the number of scientific publication since 2000 as well as explosive growth in the number of citations to cellulose biomedical materials in recent years, as shown in Figure 1. A number of good review articles have highlighted the potential applications of cellulose materials.[4,15,16,18–29] To our knowledge, these review articles are limited to the chemistry, preparation, properties, and various applications of cellulose materials, although interest in cellulose biomedical materials has increased. As the abundance of available published articles on cellulose-based materials for biomedical applications increases, here we focus on some examples of more recently investigated advanced nanocellulose materials [viz., cellulose nanocrystals (CNCs), BNC, and cellulose nanofibrils (CNFs)] in an attempt to summarize the various strategies and highlight areas where material scientists can make significant headway in the field.

## ADVANCED NANOCELLULOSE MATERIALS FOR BIOMEDICAL APPLICATIONS

### Medical Implants

***In Situ* Softening Cortical Implants.** Neural interfaces, also called *brain–computer interfaces*, bridge the central nervous system to the outside world.[30] Such interfaces hold great potential for restoring neural functions to persons with paralysis and, thus, for improving the human health of those with central nervous system disorders and our understanding of the brain. Neural interfaces are able to record neural signals from individual neurons or small groups of neurons in the brain. The commonest examples of materials being used for conventional neural interfaces are silicon, titanium, platinum, gold, iridium oxide, glassy carbon, and stainless steel.[31] The widespread use of such cortical implants has so far stifled long-term neural recording because of glia encapsulation at the electrode/tissue interface and neuron death near the surface of the implanted electrode. It has been supposed that the mechanical mismatch between the implanted electrode and the brain tissue plays a significant role to in the cell-mediated inflammatory response. As mentioned before, most neural interfaces are made from metals that are much stiffer [Young's modulus, or storage modulus ($E'$) $\approx$ 200 GPa] than brain tissue ($E' \approx$ 10 kPa).[32] Such stiff probes are easily inserted into the soft brain tissue without buckling because of their high stiffness, but micromotion between the probes and brain tissue may significantly increase the risk of tissue damage and trigger an immune response that can result in the formation of an insulting cellular sheath (gliosis), a chronic reactive biological response to the foreign probe; this leads to death of neurons and the encapsulation of the probe.[33–37] As the glial sheath forms around chronically implanted electrodes, the ability to record neuron activity is diminished within months until the electrode becomes inoperable.[38,39] It has been suggested that this sheath may not form or will be reduced in thickness if the mechanical properties of the probe closely match the mechanical properties of the surrounding brain tissue. To facilitate this, cortical implants with soft polymers (e.g., polyimide and parylene) have been developed;[40–43] this reduces the probe stiffness by approximately two orders of magnitude compared to silicon. However, as the $E'$

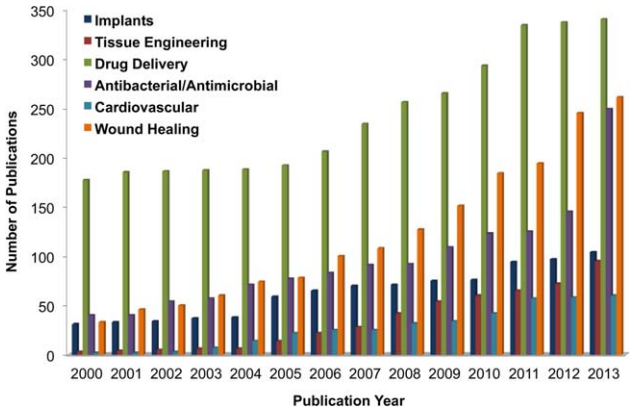


**Figure 1.** Numbers of publications in the period 2000–2013 on cellulose materials for biomedical applications. They were analyzed with the topic keywords *cellulose, implants, tissue engineering, drug delivery, antibacterial/antimicrobial, cardiovascular,* and *wound healing* in SciFinder. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

value of brain tissue is about 10 kPa[44,45] and soft polymer-based probes have a modulus of about 2–5 GPa, the mechanical mismatch is still considerable. Furthermore, the overall stiffness of these polymer-based probes is too low to penetrate the brain without buckling, unless stiff backbones[40] or gel-filled microfluidic channels are used.[43] For further details on the development, successes, and challenges of polymer-based cortical implants for neural interfacing applications, readers are referred to a very recent review by Jorfi *et al.*[46]

Recently, material scientists have developed a new class of *in situ* softening neural interfaces (physiologically responsive) as substrates for brain implants to improve the microelectrode biocompatibility and to minimize inflammatory response. These smart softening neural interfaces are stiff enough to be easily implanted into the brain but subsequently soften under *in vivo* conditions to closely match the stiffness of the brain tissue; this, thereby, minimizes the neuroinflammation response. Capadona, Rowan, Weder and colleagues[6,32,47–56] developed a new class of biologically inspired, mechanically adaptive cellulose nanocomposites (NCs) that can controllably and selectively be switched between stiff and soft states. The design of these materials was inspired by the architecture of the sea cucumber dermis. These invertebrates have the fascinating ability to rapidly and reversibly switch the stiffness of their skin (from ~5 MPa under normal conditions to ~50 MPa under threat; Figure 2).[57] This design is achieved through an NC that relies on stiff collagen fibers dispersed throughout a soft fibrillin matrix.[58–61] The sea cucumber dermis behavior offers a rich source of inspiration for the development of a series of polymer NCs with switchable stiffnesses. Because of the abundance of surface hydroxyl groups, CNCs strongly interact with each other through hydrogen bonding and/or van der Waals' forces, but exposure to hydrogen-bond-forming liquids (i.e., water) efficiently reduces CNC–CNC interactions because of competitive hydrogen-bonding or interfacial interactions with intermolecular van der Waal's forces; this feature was exploited to create water-responsive, mechanically adaptive materials.

Capadona and colleagues[6,47] developed the first generation of mechanically adaptive cellulose-based NCs. In this system, CNCs isolated from tunicate sea creatures were integrated into a rubbery ethylene oxide–epichlorohydrin copolymer matrix. $E'$ increased with CNC loading from about 3.7 MPa for the neat polymer to about 800 MPa at a CNC content of 17% v/v. These NCs exhibited a significant reduction in stiffness upon exposure to water ($E'$ decreased from about 800 to about 20 MPa for an NC comprising 17% v/v CNCs). Unfortunately, the stiffness of the most rigid ethylene oxide–epichlorohydrin/CNC NC was lower (~800 MPa) than desirable for the targeted fabrication of electrodes that could be inserted into the brain through the pia mater, the membrane surrounding the brain. Therefore, the second generation of mechanically adaptive materials was designed on the basis of an amorphous polymer, poly(vinyl acetate) (PVAc), and CNCs isolated from tunicates as reinforcing fillers.[62,63] These adaptive NCs displayed a dual responsive behavior. Upon exposure to physiological conditions, the materials underwent a phase transition (water plasticized the matrix and lowered the glass-transition temperature); in addition, the CNC network lost its load-transfer capabilities because of the loss of hydrogen bonding. This set of adaptive materials exhibited a mechanical contrast of three orders of magnitude upon exposure to physiological conditions [$E' = 5.1$ GPa for a dry NC and 12 MPa for artificial cerebrospinal fluid (ACSF)–swollen NC with 16.5% v/v CNCs]. One drawback of the PVAc/CNC NCs is their very significant water or ACSF take-up (~70–90% w/w for materials with 16.5% v/v CNCs at 37°C); this may cause the delamination of the multilayer electrode structure and excessive trauma to the surrounding tissue. The introduction of CNCs isolated from cotton into PVAc alleviates this problem.[50] PVAc/CNC NCs exhibit a large mechanical contrast (4.2 GPa–5 MPa) upon exposure to emulated physiological conditions but swell only modestly (ca. 30% w/w).

Building on this research, Hess *et al.*[64] quantified the benefits of this family of physiologically responsive NCs as substrate materials in the fabrication of variable-stiffness microstructures. In this study, model microprobes consisting of 12.2% v/v PVAc/CNC NCs were created with a lithographic technique, with the goal of implanting them into rats to study the inflammatory response (Figure 3). It was found that the micromachined structures displayed a reversible and switchable modulus comparable to bulk samples, with an $E'$ of about 3.4 GPa (in the dry state), which was reduced to about 20 MPa (in the wet state). In contrast to the bulk NCs, microstructural materials switched with a higher speed from stiff to soft in about 5 min at room temperature. Harris *et al.*[65] reported the first *in vivo* application of these mechanically switchable materials as substrates for penetrating brain implants. Figure 2(A,iii) shows that microprobes fabricated from 15% v/v PVAc/CNC NCs could readily be inserted through the pia mater into the cerebral cortex of a rat without the need for assistive devices, whereas reference probes (neat polymer) buckled before they could be inserted into the cortical tissue. *Ex vivo* studies confirmed that the stiffness of initially stiff NCs rapidly decreased when they were implanted into the rodent brain to more closely match the brain tissue [Figure 2(A) (iv)]. Furthermore, they found that adaptive

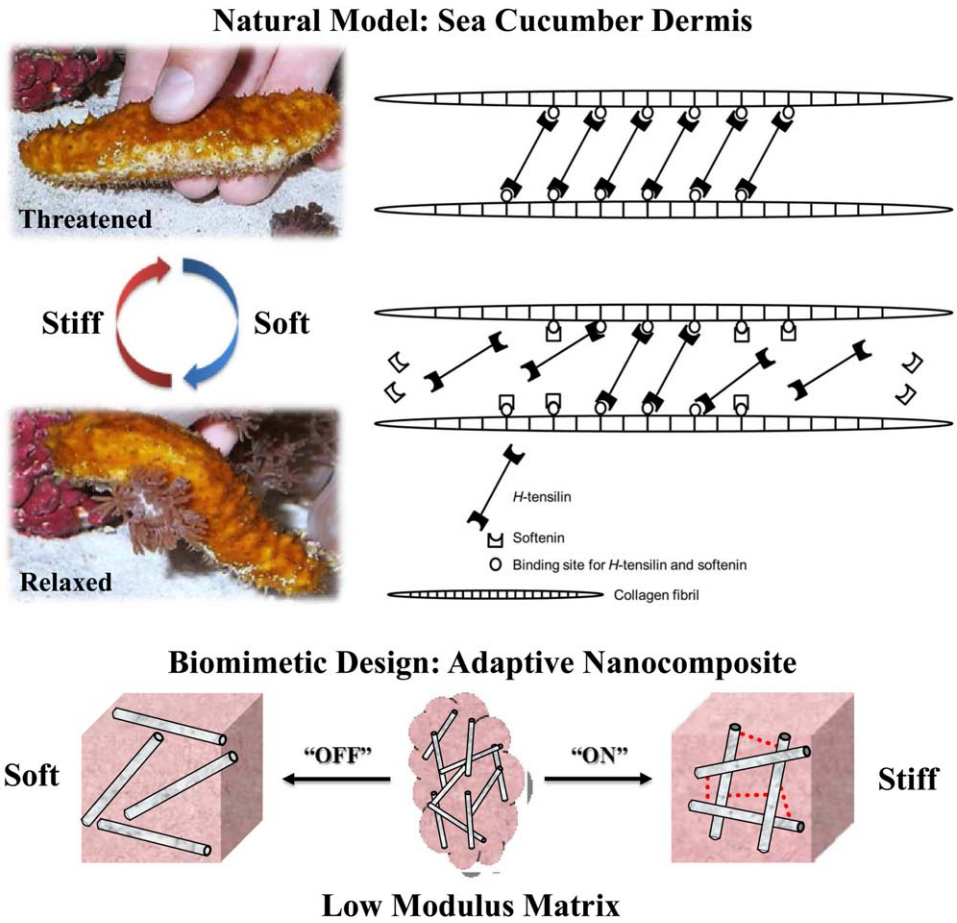


**Figure 2.** Top left: Pictures of a sea cucumber in the threatened (stiff) and relaxed (soft) state. Top right: Hypothetical model of the stiffness change mechanism in the sea cucumber dermis. Top right figure reproduced with permission from ref. 61. Copyright 2014 PLOS. Bottom: Simplified schematic representation of the switching mechanism found in the sea cucumber dermis and used in mechanically adaptive cellulose-based NCs. A soft matrix is reinforced with rigid particles, whose interactions are moderated by a chemical agent. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

microelectrodes implanted into a rat cortex for up to 8 weeks increased the cell density at the electrode–tissue interface. This demonstrated for the first time the feasibility of this novel family of mechanically adaptive cellulose-based materials for potential intracortical microelectrode applications. In another in-depth histological study, Harris *et al.*[66] investigated the effects of mechanical mismatches between the electrodes and cortical tissue with such mechanically adaptive NCs as the substrate. This body of work showed that the neuronal nuclei density within 200 $\mu$m of the implant at 4 weeks postimplantation was significantly greater for the compliant NCs compared to the rigid wire. More recently, Nguyen *et al.*[67] completed a more comprehensive histological evaluation of the neuroinflammatory response to PVAc/CNC NC implants. At 16 weeks postimplantation, Nguyen *et al.* demonstrated the near complete attenuation of microglia activation and the absence of any appreciable neuron loss surrounding PVAc/CNC implants compared to PVAc-coated microelectrodes [Figure 3(A)].

To improve upon the current-generation PVAc/CNC NCs, it is desirable to raise the initial stiffness of the material to above 5 GPa, as this ensures the reliable insertion of the cortical microelectrodes and eases the fabrication of smaller brain probes. Jorfi *et al.*[54] explored mechanically adaptive NCs based on poly(vinyl alcohol) (PVA) as the matrix and CNCs derived from tunicates or cotton as the filler. This design was based on the hypothesis that the use of a polar glassy polymer that promotes significant matrix–filler interactions would result in stiffer materials than previously used matrices.[63] For example, in the dry state at 25°C, $E'$ increased from 7.3 GPa for the neat polymer to about 9.0 or 14 GPa when 16% v/v CNCs were incorporated. The stiffness of this material was greatly reduced upon exposure to simulated physiological conditions, ACSF at 37°C; this caused a drastic drop in $E'$ to about 1 MPa.

To date, it was also shown that antioxidative treatment is a strategy for temporally mitigating the neuroinflammatory response to intracortical microelectrodes.[66,68] Toward this end and to explore whether the combination of two independently effective mechanisms, softening and antioxidant release, would lead to a synergistic effect in the reduction of neuroinflammation at the intracortical microelectrode–tissue interface, Potter, Jorfi *et al.*[52] recently developed a first series of curcumin-releasing mechanically adaptive implants based on PVA and

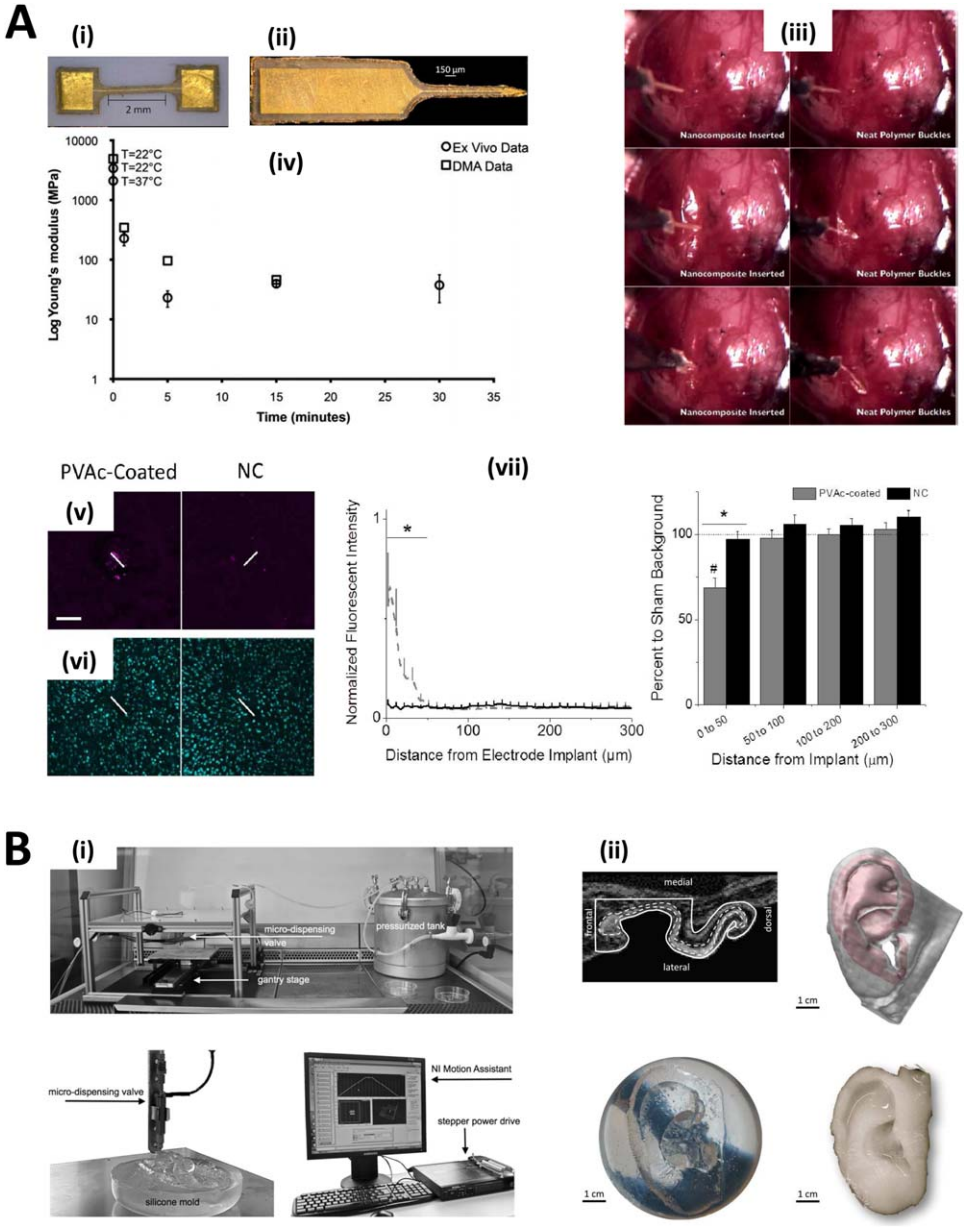


**Figure 3.** (A) (i) Micromachined dogbone structure with lithographically defined Ti/Au pads and trace and (ii) a laser-micromachined PVAc–CNC brain probe with a lithographically defined Ti/Au electrode. Parts i and ii reproduced with permission from ref. 64. Copyright 2011 IOP Publishing. (iii) Snapshots of a movie that show insertion attempts of microprobes consisting of a neat polymer and mechanically adaptive NC implants. (iv) Plot showing log $E'$ of PVAc/CNC NCs as function of the exposure time to ACSF or the implantation time in the rat cortex. Data were acquired by either a dynamic mechanical analysis (DMA; DMA data, open squares; bulk materials) with a submersion clamp and exposure of the sample to ACSF preheated to 37°C or mechanical tests of microprobes that were implanted into the rat cortex for the time indicated and that were explanted for microtensile testing (*Ex vivo* data, open circles). The *x* axis indicates the time of exposure to either ACSF or implantation in the rat cortex, respectively. Parts iii and iv reproduced with permission from ref. 65. Copyright 2011 IOP Publishing. (v) IHC staining of CD68 for activated microglia showing increased expression surrounding the PVAc-coated implants compared to the PVAc/CNC NC implants (NC) (v, vii). (vi) Staining of the neuronal nuclei and (viii) quantification of neuron counts surrounding the NC implants from 0 to 50 μm (scale bar = 100 μm). Reproduced with permission from ref. 67. Copyright 2014 IOP Publishing. (B) Biofabrication of a patient-specific ear-shaped BNC implant. (i) The bioprinter consists of a high-precision motion system and a microdispensing system. (ii) Transverse slice isolated from a spoiled gradient–echo MRI scan of the volunteer's left ear. A negative silicone mold was used to guide the bacteria to reproduce the large-scale features of the outer ear. The 3D BNC implant prototype was fabricated in the shape of the whole outer ear. Reproduced with permission from ref. 82. Copyright 2013 Elsevier. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

CNCs derived from tunicates. An *in vivo* study in rats showed that after 4 weeks, the new curcumin-releasing, softening implants promoted higher neuron survival and a more stable blood–brain barrier than the neat PVA controls, but the benefits of the curcumin release were lost after 12 weeks, where the antioxidant-releasing compliant materials caused no statistically significant differences in the neuronal density distribution profiles, namely, that of the PVA reference. This study showed that cellulose-based bionanocomposites could be combined with bioactive molecules to build multicomponent, biologically relevant biomedical implants for specific medical applications.

**Soft-Tissue Implants and Cartilage Replacements.** The discovery of suitable biomedical materials for soft-tissue replacement and reconstruction applications is an important aspect for the development of medical implants that not only have similar mechanical characteristics as the tissue it replaces but also show improved biocompatibility, nonthrombogenic, sterilizability, durability, life span, lesser degrees of calcification, and good processability for ease of manufacturing.[69] The implant should be biocompatible with the host tissues in terms of chemical, mechanical, surface chemistry, and pharmacological properties. The fibrillar network of nanocellulose materials such as BNC offers high tensile mechanical properties to the material[70] and a hydrogel-like behavior as cellulose interacts with surrounding media, such as water.[19] In addition, BNC specifically is nondegradable under physiological conditions, and it has been also shown to be biocompatible.[71–73] Nondegradable biomaterials provide durable mechanical properties and long-term chemical stability in contrast to their counterparts, degradable ones. All these key characteristics make BNC an exciting biomaterial candidate for pharmaceutical and biomedical applications,[15,19] including blood vessel,[74,75] meniscus,[76,77] and articular cartilage tissue engineering.[78] Interconnected porosity is another key requirement in scaffold applications; it allows chondrocytes to penetrate and migrate throughout the biomaterial. Although BNC is known to be impenetrable by cells because of its small pore size,[71,79] this issue has been resolved in recent years by several approaches, which successfully synthesize BNC scaffolds with large pore size and allow the seeded cells to penetrate throughout the scaffold.[80,81]

Therefore, in recent years, there has been an increasing amount of interest in the development of nanocellulose-based biomaterials for soft-tissue replacement and reconstruction. Bodin *et al.*[76] compared the mechanical properties of BNC gels with collagen meniscal implants and real pig menisci harvested from pigs. They found that the mechanical properties of the BNC gel were similar in magnitude to the ones of pig menisci. The $E'$ value of the BNC gel was measured to be 1 MPa; this was 100 times higher than the one of the collagen material, 0.01 MPa in tensile load. The combination of inexpensive materials, controlled meniscus shape design, and promising cell migration makes BNC materials an attractive candidate for future meniscus implant applications. In a recent study, Nimeskern *et al.*[82] designed and fabricated an ear-shaped BNC prototype material, which was produced from a negative ear mold with a MRI scanning technique [Figure 3(B)]. It was reported that the mechanical properties of BNC materials could be regulated by the effective cellulose contents. This study confirmed that BNC is a promising material with appropriate mechanical properties for ear cartilage replacement; it, thereby, may be used to create patient-specific ear shapes.

**Drug Delivery**

Recent development in materials science and chemistry in biomedical applications has led to the creation of various drug-carrier systems and approaches. Cellulose has a well-documented history of successful use in U.S. Food and Drug Administration approved drugs/products. For example, cellulose acetate (CA) has been used successfully in several HIV drugs, two antibiotics, a pain reliever, and five flavonoids, just to name a few.[83] Also, hydroxypropyl methylcellulose has been used in oral drug-delivery formulations. One of the key purposes of using cellulose as excipients in drugs is to control the rate of drug release and achieve the right drug concentration. This natural polymer can also be crosslinked into hydrogels because of its affinity toward water. Additionally, cellulose and cellulose derivatives pass through the human body safely, and some of the derivatives can be broken down digestive enzymes into natural metabolites in the gastrointestinal tract.[84]

Several drug-delivery systems based on nanocellulose materials for various pharmaceutical applications have been used in recent years.[85–97] In one study, Trovatti *et al.*[87] used BNC membranes as systems for topical release of lidocaine. *In vitro* drug-release studies in a phosphate buffer solution (pH 7.4) at 32°C showed a burst release profile in which more than 90% of the total drug was released in the first 20 min. The therapeutic applicability of three different BNC–lidocaine systems (BNC, a gel, and an aqueous solution) were evaluated *in vitro* with human epidermis. It was found that the permeation rate of lidocaine in the BNC membranes was significantly lower than those obtained with the other two systems (gels and aqueous solutions).[87] In another study, Müller *et al.*[94] investigated BNC as potential drug-delivery system for proteins with serum albumin. They found that the freeze-dried BNC samples showed a lower uptake of protein than the native BNC samples. Finally, Müller *et al.* showed that the biological stability of albumin was maintained during materials processing. In 2012, Dash and Ragauskas[89] adapted the concept of drug-delivery carriers based on nanocellulose materials for amine-containing drugs. In this study, a periodate oxidation Schiff's base condensation reaction was used to graft a spacer molecule, $\gamma$ aminobutyric acid, to CNCs. Then, to achieve a slow and rapid release profile of the targeting moiety, syringyl alcohol was used as an aromatic releasable linker and attached to it.

Chang and Wang[93] recently developed hydrogen peroxide ($H_2O_2$) and oxygen ($O_2$) releasing microfibrillated cellulose (MFC) based NCs that modulate the growth of mammalian cells. In this investigation, calcium peroxide (CPO) was embedded into highly porous MFC NCs to produce $H_2O_2$, whereas catalase was added to convert the generated $H_2O_2$ to $O_2$ under physiological conditions [Figure 4(A)]. *In vitro* cell culture studies for 1 and 5 days showed that cell attachment was decreased and cell proliferation was delayed in MFC/CPO NCs, clearly because of the toxicity of $H_2O_2$ and/or hydroxyl radicals. On

the other hand, cell survival was significantly increased in the case of NCs in contact with CPO and catalase; this indicated the effective conversion of $H_2O_2$ to $O_2$, which provided nutrition for cell growth and proliferation for up to 5 days.[93]

Carboxymethylcellulose (CMC) is another attractive cellulose material that has attracted increasing interest in recent years in the drug-delivery field.[95–97] Recently, Weng *et al.*[95] reported CMC microspheres with adjustable anticancer drug-release properties for potential arterial embolization applications. The as-prepared microspheres loaded with the anticancer drug doxorubicin showed a burst release profile in first 8 h followed by a plateau release over a 24-h period under physiological conditions. Although *in vivo* histological studies in the kidneys of rabbits after 6, 7, and 73 days of embolization revealed that the microspheres were biodegradable with only a mild tissue response [Figure 4(B)],[95] more long-term animal studies are needed for a better evolution of the microsphere degradation effects for future medical applications. In 2011, Ernsting *et al.*[98] developed docetaxel (DTX)-loaded CMC-based nanoparticles (NPs) for enhanced cytotoxicity against cancer cells. These spherical NPs (~120 nm) released DTX at a slow and controlled rate of 3.8 w/w % per day (100% in 3 weeks). *In vitro* studies showed an enhanced cytotoxicity by 2–40 fold compared to free DTX against cancer cells, presumably because of the slow release profile. Further *in vivo* antitumor evolution of these DTX-releasing CMC NPs exhibited 90% tumor growth inhibition compared with that of the native DTX.[96] In another study by Wen and Oh,[97] dual-stimuli-responsive CMC-based nanogels were reported as potential intracellular anticancer drug-delivery carriers. In this study, oligo(ethylene oxide)-containing methacrylate (OEOMA) was polymerized in the presence of CMC and a disulfide-labeled dimethacrylate with free-radical crosslinking polymerization [FRCP; Figure 4(C)]. These nanocarriers allowed drug release in response to both acidic pH and thiol reducing agents. Furthermore, the *in vitro* intracellular release applicability of these nanocarriers containing the anticancer drug doxorubicin was confirmed with HeLa cancer cells.[97]

### Wound Healing

One of best known clinical applications of cellulose materials, especially BNC, is as a topical material in wound healing. Burns are very complex injuries, and they cause extensive damage to skin tissue. According to the results of many studies in the field of wound healing, BNC has been shown to be a superior candidate for conventional wound-dressing materials. BNC-based dressing materials, such as XCell, Bioprocess, and Biofill, are already commercially on the market for topical application in wound healing.[15] The applications of BNC-based materials for skin-tissue repair have been reviewed already elsewhere.[17] Herein, we summarize the most recent examples of advanced nanocellulose-based materials used in wound-healing applications.

The results of many studies indicate that topical applications of BNC improve the healing process of burns and chronic wounds. Czaja *et al.*[19] used BNC membranes to treat patients with severe second degree burns [Figure 5(A)]. This study showed that the skin of the patients whose burns were covered with a BNC membrane healed faster than the wounds of patients who received conventional wound dressings. Czaja *et al.* discovered that never-dried BNCs showed remarkable conformability to various body counters, maintained a proper water balance, and significantly reduced wound pain. More recently, animal studies by Fu *et al.*[99] also confirmed the faster tissue regeneration, better healing effect, and lower inflammatory response of BNC-based dressing materials.[99]

Until now, gauze dressings have been the most widely used clinical wound dressings. In a recent study by Fu *et al.*,[100] BNC has been used as a potential skin-tissue repair material *in vivo* to replace conventional gauze dressings.[100] Pathological studies showed better and faster healing effects and less inflammatory response in the thick-BNC group after 14 days compared to the other groups (i.e., the control and thin-BNC groups). Histological studies exhibited significant tissue regeneration, capillary formation, and cell proliferation in the wound area in the thick-BNC group on day 7 compared to the other groups [Figure 5(A)]. On the basis of this study, the wound healing was faster in the thick-BNC group than in the thin-BNC groups. This indicated the influence of the BNC thickness when it acted as a wound-dressing material.[100] In a similar investigation, BNC wound-dressing materials were compared to two different commercial dressings, Vaseline gauze and Algisite M, in a rat model.[101] This study showed that BNC-dressed animals had more rapid wound healing on day 14 without any evidence of toxicity compared to other groups; this confirmed the efficacy of BNC dressing materials for clinical applications.

CMC also has potential for use in wound-dressing materials. Fan *et al.*[102] showed that a *in situ* crosslinked hydrogel made through the crosslinking of oxidized carboxymethylcellulose (OCMC) and carboxymethyl chitosan were capable of healing second-degree burns without any significant adverse reactions at 14 days postwounding with a rat model. This work opens a new avenue for the design and development of new wound-dressing materials with different types of cellulose toward wound-healing applications. Taken together, nanocellulose material, especially BNCs, is a promising biopolymer for skin-tissue-repair applications. The many advantages of nanocellulose-based materials, along with a rapidly developing recent interest in natural medical polymers, will eventually lead to a new biomaterial for use in a wide variety of medical applications, especially in skin-tissue repair.

### Tissue Engineering

Another area of innovative and exciting potential for nanocellulose material use is tissue engineering.[103] Thanks to the unique three-dimensional (3D) network formed by cellulose and its mechanical properties and potential biocompatibility, cellulose is an ideal material candidate for a variety of tissue engineering applications.[78,104,105] Although, diverse cellulose species have been used to fabricate bionanocomposites containing hydroxyapatite (HA), it seems that BNC is the most promising material for potential tissue engineering, mostly because of its low cytotoxicity and high porosity. Therefore, the majority of recent studies for tissue engineering applications have used BNC, a cellulosic material with unique properties among other

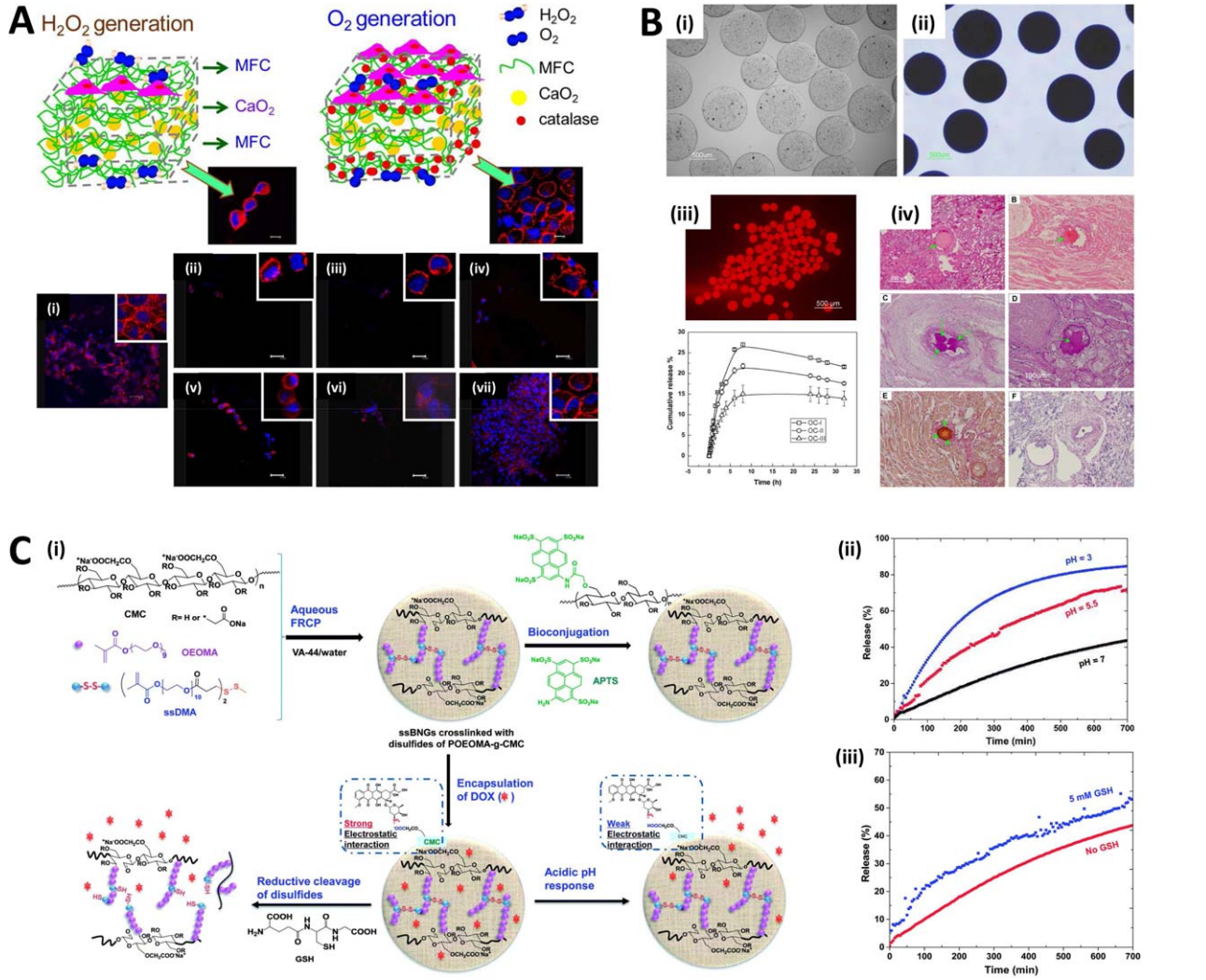


**Figure 4.** (A) Cell morphology of the (i) pristine MFC. MFC/CPO-*x* with different CPO concentrations: (ii) 5, (iii) 10, and (iv) 15 wt %. MFC/[CPO + catalase] with different CPO concentrations: (v) 5, (vi) 10, and (vii) 15% w/w. Scale bar = 40 $\mu$m. Reproduced with permission from ref. 93. Copyright 2013 American Chemical Society. (B) Optical microscopy images of the OCMC/CCN microspheres: (i) original and (ii) stained with Evan's blue. (iii) Fluorescent image of doxorubicin-loaded OC-II microspheres (top) and doxorubicin release profiles of the OCMC/CCN microspheres in 0.01 M PBS at 37°C (bottom). (iv) Histological sections of the rabbit kidneys after embolization. Reproduced with permission from ref. 95. Copyright 2013 Elsevier. (C) Synthesis of stimuli-responsive CMC-based nanogels by aqueous FRCP. (ii) Release of DOX in aqueous buffer solutions at different pHs of 3, 5.5, and 7. (iii) Release of DOX in aqueous buffer solutions at pH 7 with and without 5 m*M* GSH. DOX, doxorubicin hydrochloride; GSH, glutathione; VA-44, 2,2′-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride; APTS, 8-aminopyrene-1,3,6-trisulfonic acid trisodium salt; ssDMA, dithiopropionyl poly(ethylene glycol) dimethacrylate; ssBNG, dual stimuli-responsive bionanogels. Reproduced with permission from ref. 97. Copyright 2014 Royal Society of Chemistry. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

biomaterials that is used in tissue engineering scaffolds. Watanabe *et al.*[104] investigated the biocompatibility of BNC in cell cultures. In this study, a new mammalian cell culture substrate was developed with an *Acetobacter aceti* produced BNC. In this research, the authors showed that a serum-soaked BNC membrane was an effective substrate for use in tissue engineering.

The topography and architecture of tissue engineering scaffolds (i.e., surface porosity, fiber network structure, surface topology, and fiber density) are essential components that influence the cell–biomaterial interaction and, therewith, the cell behavior. In a recent investigation, Berti *et al.*[106] reported that immortalized human vein endothelial cells presented a different behavior when they were cultured on two distinctly different BNC surfaces. The results show that although both BNC surfaces maintained viable endothelial cells, the porous BNC surface sustained more viable cells compared to the entangled surface at 20 days of culturing; this suggested that the fiber network arrangement or density was the responsible factor for endothelial cell differential behavior. Highly porous cellulose scaffolds based on pectin, CMC, and MFC were also fabricated by a lyophilization method.[107] *In vitro* studies exhibited highest cell viability for these porous composite scaffolds.

Polymer-based scaffolds for bone tissue engineering often fail to maintain high mechanical strength stability and mostly require

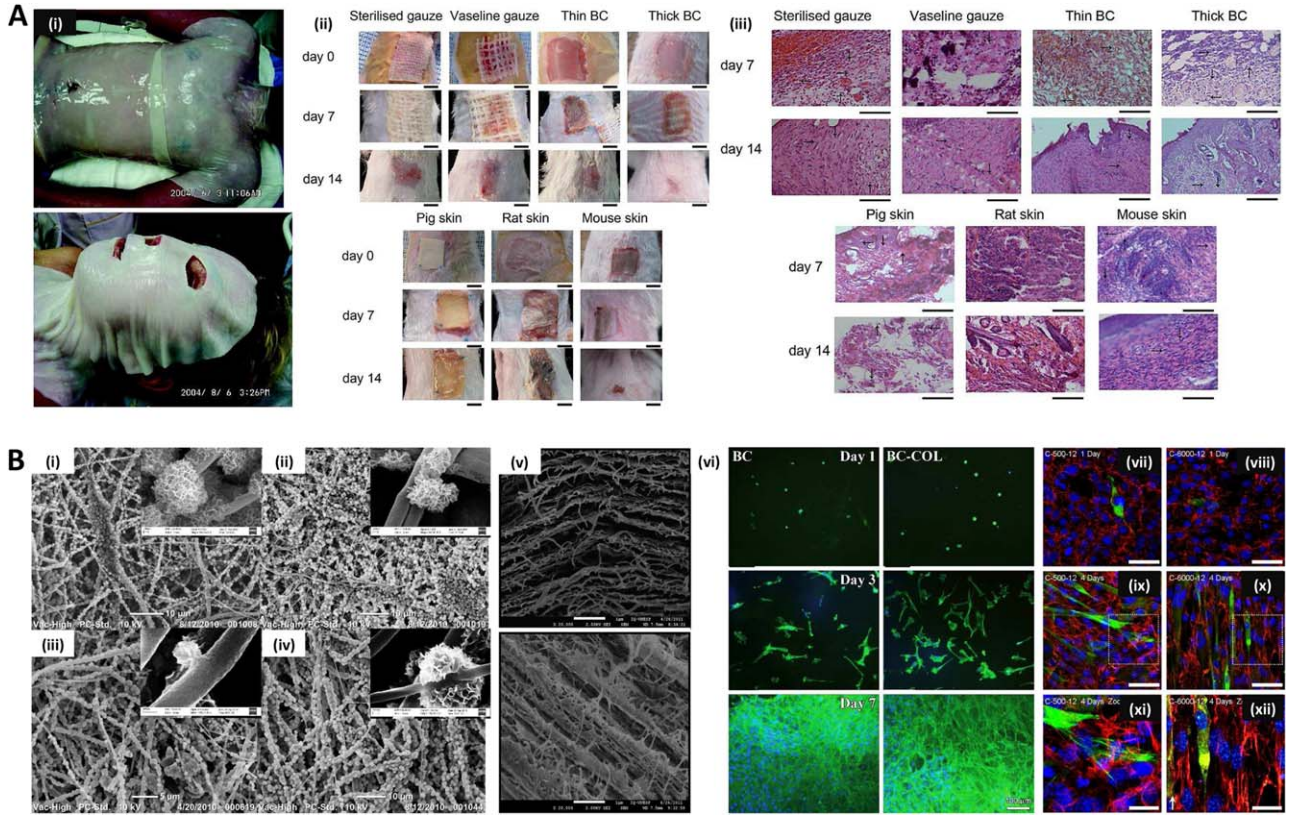


**Figure 5.** (A) Applications of cellulose materials in wound healing: (i) Never-dried BNC membranes with different shapes and sizes applied on a wounded torso and face. Reproduced with permission from ref. 19. Copyright 2007 American Chemical Society. (ii) Macroobservations of the full-thickness skin lesion and dressing experiments in mice (scale bar = 5 mm). (iii) Light microscopy images of the pathological sections in mice experiments (scale bar = 100 mm). Reproduced with permission from ref. 100. Copyright 2012 Royal Society of Chemistry. (B) Applications of cellulose materials in tissue engineering: (i–iv) Scanning electron microscopy images of mineralized scaffold with CMC treatment: (i) 45-min RC and (ii) 24-h RC treated with procedure 1 and (iii) 45-min RC and (iv) 24-h RC treated with procedure 2. Reproduced with permission from ref. 113. Copyright 2011 American Chemical Society. (v) SEM cross-sectional images of the BNC membrane (top) and BNC–COL composite (bottom) at 20,000×. (vi) Fluorescence images of osteoblast cells cultured on BNC (left) and a BNC–COL composite (right) at 1, 3, and 7 days. The green fluorescence (Alexa Fluor 488-conjugated phalloidin) shows that the actin cytoskeleton, and the blue fluorescence (DAPI DNA stain) shows the cell nuclei (scale bar = 100 $\mu$m). Parts v and vi reproduced with permission from ref. 116. Copyright 2012 Royal Society of Chemistry. (vii–xii) Fluorescence images of C2C12 myoblast cells stained for myosin heavy chains (green), fibronectin (red), and nuclei (blue) on (vii–ix) low-oriented and (x–xii) high-oriented CNCs surfaces [scale bar = 50 μm (vii–x) or 20 μm (xi and xii). Reproduced with permission from ref. 119. Copyright 2013 Elsevier (BC, bacterial cellulose). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

chemical crosslinking to stabilize such scaffolds. Therefore, it is often a challenge to create load-bearing scaffolds for bone tissue engineering applications. The key attractive advantages of cellulose for bone tissue engineering are its mechanical properties (to tolerate local forces) and biocompatibility (to integrate with host tissue without immune responses). Recently, Kumbar and coworkers,[108,109] in a series of contributions, developed mechanically competent cellulose scaffold materials for bone tissue engineering applications. In this study, CA and ethyl cellulose microspheres were fabricated via an oil-in-water emulsion–solvent evaporation technique and were then sintered together into 3D porous scaffolds with a solvent–nonsolvent sintering approach. These scaffolds showed mechanical properties in the midrange of human trabecular bone and were superior to several current polymer-based bone tissue engineering scaffolds with similar pore properties under both dry and physiological (wet) conditions. It was found that the surface functionalization of the scaffolds with collagen type I nanofibers enhanced the surface area and bioactivity of the scaffolds. These nanocellulose-based scaffolds functionalized with collagen exhibited better human osteoblast adhesion, proliferation, and alkaline phosphatase expression compared to control PLGA-based scaffolds and, thereby, may serve as potential alternatives to other polymer-based scaffolds for bone tissue engineering.[109]

HA is a well-known bone replacement biomaterial because of its properties, including its biocompatibility with the human body, bioactivity, osteoconductivity, and noninflammatory properties. HA/cellulose NCs have been extensively investigated in recent years for potential tissue engineering applications.[110–115] For example, HA/BNC NC scaffolds were prepared by a biomimetic approach for bone-healing applications.[114] In this study, CMC was adsorbed onto the BNC surface to induce nucleation of calcium-deficient HA and then treated with

simulated body fluid (SBF) over a one-week period. *In vitro* experiments indicated that the presence of calcium-deficient HA crystals on the BNC surfaces increased cell attachment and the alkaline phosphatase (ALP) activity of bone cells. In a similar approach, Rodriguez *et al.*[113] reported electrospun regenerated cellulose (RC)-based scaffolds with the ability to nucleate bioactive calcium phosphate crystals for potential bone-healing applications. Similarly, CMC was adsorbed on the RC scaffolds in the presence of $CaCl_2$ and was then treated with SBF to produce Ca–P crystals. Rodriguez *et al.* explored two different ways to modify the cellulose surface: (1) exposure to CMC and $CaCl_2$ at room temperature for 24 h and then treatment with SBF solution and (2) exposure to CMC and $CaCl_2$ at 80°C and pH 8 for 2 h and then treatment with SBF solution. It was shown that HA-like biomimetic crystal growth occurred on the surface-modified *in vitro* cellulose scaffolds with both CMC treatment methods [Figure 5(B)]. Calcium-deficient HA/BNC NC were also synthesized by a biomimetic mineralization process where alkaline treatment and $Ca^{2+}$ activation were introduced before the biomimetic mineralization process.[112] The mineralization efficiency was improved in case of NCs treated with alkaline compounds compared with those without alkaline treatment, presumably because ion exchange between $Na^+$ and $Ca^{2+}$ could easily occur.

Even though the as-mentioned studies showed excellent properties of BNC for tissue engineering applications, several studies have been made to improve the biological properties of BNC for potential medical applications, including tissue engineering and regeneration. For instance, Saska *et al.*[116] developed composites based on BNC and type I collagen (COL) for potential bone tissue engineering, in which collagen was homogeneously and covalently introduced into the BNC network. *In vitro* cell culture experiments with osteogenic cells revealed that collagen I did not affect cell adhesion and proliferation or the cell morphology [Figure 5(B)]. BNC–COL composites showed higher values of ALP protein *in vitro* at day 17; this is an early marker for osteoblastic differentiation. Heparin (Hep) was also hybridized with the BNC network to build Hep–BNC nanofibrous scaffolds with anticoagulant properties for potential use in vascular tissue engineering.[117]

Researchers have also recently explored the potential of CNCs to provide nanoscale cues for tissue engineering applications.[118,119] Until today, however, just two attempts have been reported to use the unique properties of CNCs for tissue engineering. In the first investigation, high-aspect-ratio CNCs derived from the tunicate *Halocynthia roretzi* were deposited onto glass substrates by a simple spin-coating technique. It was found that myoblasts (muscle cells) were able to effectively sense the CNC surface topography and orientate relative to the bulk direction of the CNC orientation. In a more recent study by Eichhorn *et al.*,[119] CNCs extracted from the marine invertebrate *Ascidiella aspersa* were oriented on glass coverslips with a similar spin-coating method. CNCs with nanoscale dimensions were shown to induce a similar guidance response in C2C12 skeletal muscle myoblasts but also promoted the degree of myoblast fusion, terminal differentiation, and template deposition of an oriented fibrillar extracellular matrix [Figure 5(B)]. These studies have indicated the potential of CNCs as an ideal candidate for tissue engineering scaffolds. Specifically, the high-aspect-ratio, nanoscale dimensions, biocompatibility, and low cytotoxicity of tunicate CNCs could be useful for providing nanoscale cues for the *in vitro* culturing of highly oriented tissues, such as skeletal muscle.

**Antibacterial/Antimicrobial Activity**

It should be noted that cellulosic materials do not intrinsically present antibacterial and/or antimicrobial properties. Because of the enormous potential of cellulose materials, it is of great interest to develop novel functional cellulose-based biomaterials by introducing antibacterial/antimicrobial activities through the functionalization and/or incorporation of antibacterial/antimicrobial agents. For instance, antimicrobial nanocellulose-based materials have been obtained by the incorporation of *N*-halamine[120] and nanocurcumin[121] into the cellulose network and by the chemical grafting of aminoalkyl groups,[122] 2-benzyl-4-chlorophenol,[123] L-cysteine,[124] and diclofenac[125] onto the surface of the cellulose backbone.

In one study, an electrospinning technique was successfully used to prepare composite nanofiber fabrics with CA nanofibers and the *N*-halamine antimicrobial agent of bis(*N*-chloro-2,2,6,6-tetramethyl-4-piperidinyl) sebacate (Cl-BTMP).[120] The electrospun composite nanofiber fabrics containing Cl-BTMP showed significant antimicrobial activity against *Staphylococcus aureus* (Gram positive), *Escherichia coli* (Gram negative), and *Pseudomonas aeruginosa* (Gram negative) compared to the control solution-cast films; this was clearly attributed to the aggregation of Cl-BTMP in the solution-cast samples. More recently, Raghavendra *et al.*[121] impregnated nanocurcumin (curcumin NPs) into cotton nanocellulose fibers for potential antimicrobial applications. The cumulative release studies indicated that the fibers released all of the nanocurcumin at about 60 h. Moreover, the antimicrobial activity results show an effective antimicrobial against *E. coli* and *S. aureus* over a period of 24 h.

In 2013, Fernandes *et al.*[122] developed bio-inspired antimicrobial nanocellulose membranes by the chemical grafting of aminoalkyl groups on the surface of bacterial cellulose for biomedical applications. The aminoalkyl-grafted bacterial nanocellulose (BNC–$NH_2$) membranes were prepared in three steps: (1) hydrolysis of the silane derivative, (2) adsorption of the hydrolyzed species onto BNC nanofibrils, and (3) a chemical condensation reaction. The BNC–$NH_2$ membranes showed a significant reduction in bacterial cells for both *E. coli* and *S. aureus* after 24 h, whereas the BNC membranes without functionalization showed no reduction in bacterial viability.[122] Similarly, Caldeira *et al.*[124] reported nanocellulosic fibers with effective antibacterial properties obtained by the surface functionalization of 2,2,6,6-tetramethylpiperidine-1-oxyl radical activated cotton cellulose fibers with L-cysteine.

In a different approach, Butchosa *et al.*[126] reported cellulose-based NCs with antibacterial activity, simply by combining BNC nanofibers and chitin nanocrystals. Chitosan has been used widely as an antimicrobial agent in biomaterial applications and is produced via the deacetylation of chitin. In this study, partially deacetylated chitin nanocrystals (D-ChNCs) were

introduced into the BNC nanofiber 3D network by either *in situ* biosynthesis or postmodification. It was found that the bactericidal activity of BNC/D-ChNC NCs against *E. coli* was strongly dependent on the D-ChNC content.[126]

In the last 5 years, several approaches have been successfully developed to incorporate metal particles into nanocellulose materials for antibacterial activity, such as the physical blending of NPs with cellulose and *in situ* sol–gel formation of metal particles within cellulose materials.[127–132] For example, silver particles have been used as potential agents with a broad antibacterial activity and low presumed toxicity to coat cellulosic materials for biomedical applications. Li *et al.*[128] reported the microwave-assisted synthesis of silver/cellulose NCs with high antibacterial properties against *E. coli* (Gram-negative bacteria) and *S. aureus* (Gram-positive bacteria). The silver/cellulose NCs prepared by the formation of Ag NP through the *in situ* reduction of silver nitrate in ethylene glycol on the surface of microcrystalline cellulose (MCC).[128] Although metal NPs have been associated with remarkable antibacterial properties, safer and greener approaches using the reduction of metal slats are still limited in the literature. Recently, researchers have reported different preparations of metal-coated cellulose materials without using toxic chemical reductants such as $NaBH_4$ as a reducing agent for the metal salts.[127,129,131] For instance, Barua *et al.*[131] recently reported the preparation of copper–copper oxide (Cu–CuO) NP-coated CNFs through a green reductive technique by ethanolic extracts of the *Terminalia chebula* fruit. The Cu–CuO NP-coated cellulose materials exhibited promising antimicrobial activity against Gram-positive and Gram-negative bacteria and fungal species.

**Cardiovascular**

Cardiovascular diseases are the number one cause of death for both men and women globally. According to the World Health Organization and British Heart Foundation, cardiovascular diseases account for 30% of deaths worldwide and 42% in the Europe. Every year, thousands of patients around the world have heart bypass surgery. Because of the lack of artificial bypass implants for this purpose so far, vessels are drawn from the legs or thorax of patients. Common synthetic bypass implants made of polytetrafluoroethylene, poly(ethylene terephthalate), polyethylene, and polyurethane have been unsuccessful for cardiovascular surgery. Several research groups have developed BNC-based implants, which conform to blood and tissue compatibility, endothelization, cell ingrowth, surgical handling, and common methods of sterilization.[16] Klemm and coworkers[79,133–136] in a series of investigations have developed prototypes of BNC tubes (brand name BASYC, Bacterial Synthesized Cellulose) with different diameters; these can be used for arterial grafting applications [Figure 6(A)]. The wall of the BASYC tubes consist of BNC loaded with 90% water or more. The initial studies showed that the BNC tubes have very good surgical handling and can be sterilized in standard ways. In a follow-up *in vivo* study with rats, pigs, and sheep, the BNC tubes were successfully used to replace carotid arteries [Figure 6(A)].[134,135]

The first animal study of the carotid artery–BASYC complex on rats showed good biocompatibility and incorporation into the body without any rejection after 4 weeks.[134,136] The long-term, 1-year experimental investigations with rats showed the incorporation of the BNC-based implants under formation of neointima and the ingrowth of active fibroblasts. These long-term results with rats confirm the results of 4-week animal studies. In a later preliminary *in vivo* study with pigs, seven BNC tubular grafts were patented, whereas one BNC graft was found occluded after 3 months. Interestingly, the BNC grafts also promoted *in situ* vascular tissue regeneration.[135] These data indicated that stable BNC-based vascular conduits are possible and can be used as bioengineered, synthetic BNC-based grafts in small-diameter arteries in cardiovascular surgery.[136]

It is important to ensure a good mechanical match between the implanted device and the surrounding tissues in cardiovascular tissue replacement applications. A mechanical mismatch between the synthetic compliant grafts made of elastic polymers, such as polyester (Dacron) and expanded polytetrafluoroethylene, and the surrounding native tissue has been reported as a major factor in intimal hyperplasia and ultimate graft failure of the currently used cardiovascular graft replacements.[137] As a step toward fixing this problem, Millon *et al.*[138] reported anisotropic NCs based on PVA and BNC with a broad range of mechanical properties and a controlled degree of anisotropy suitable for potential cardiovascular tissue replacement. The mechanical properties of the PVA–BNC NC materials were closely matched with the mechanical properties of the porcine aorta within physiological range. More recently, Azevedo *et al.*[139] used cellulose and chitosan blends to fabricate small-diameter hollow tubes with a compliance that closely matched that of human coronary arteries. One can tune the mechanical properties of the cellulose/chitosan blends by simply changing the ratio between each component. Furthermore, these synthetic biopolymer-based tubes exhibited cell compatibility properties that are promising for further investigation as a potential synthetic biocompatible candidate for coronary artery bypass graft applications.

**Other Biomedical Applications**

In addition to the biomedical applications discussed previously, nanocellulose materials have also been used in other biomedical applications, such as cancer targeting,[140] cornea replacement,[141] biological detection,[142] and biology–device interfaces.[143] In one example, CNCs were used for the targeted delivery of chemotherapeutic agents to cancer cells.[140] Briefly, Dong *et al.*[140] synthesized folic acid (FA) grafted CNCs for the first time and explored *in vitro* their folate-receptor-mediated uptake by human and rat brain tumor cells. The reaction pathway scheme used for the synthesis of FA-grafted CNCs is shown in Figure 6(B). First, CNCs were labeled with fluorescein isothiocyanate (FITC) and were then conjugated with FA. FITC is necessary for the detection of the CNCs in *in vitro* cell-uptake studies. *In vitro* studies showed that the cellular binding/uptake of the FITC–CNC–FA by the folate receptor, which was overexpressed by several types of cancer cells, was significantly higher than that of the free FA. This study suggests that the FA-conjugated CNCs selectively targets the folate-receptor-positive cancer cells and are promising candidates for potential cancer targeting.[140]

In another example, Wu *et al.*[144] reported nanogel complexes made of poly(*N*-isopropyl acrylamide-*co*-butyl methacrylate)

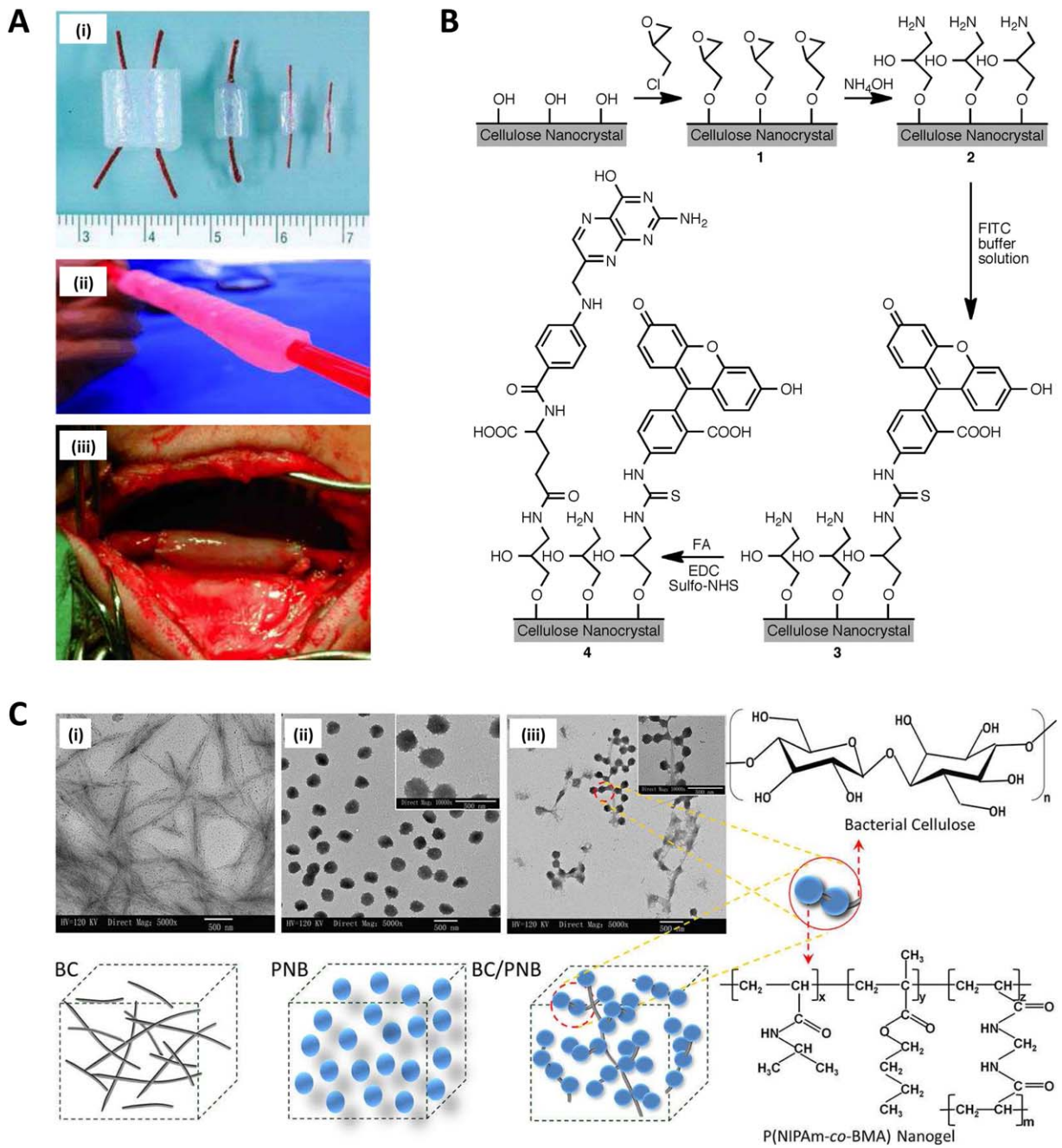


**Figure 6.** (A) Cardiovascular applications of BNC-based materials. (i) BNC tubes with different diameters (inner diameter = 0.6–6 mm) created by a matrix technology for arterial grafting applications. Reproduced with permission from ref. 16. Copyright Cambridge University Press. (ii) Long BNC tube designed with a matrix technology used as a blood-vessel implant (inner diameter = 6 mm, length = 15 cm). (iii) BNC tube as a long-segment vascular graft for the right carotid artery of a sheep. Parts ii and iii reproduced with permission from ref. 28. Copyright 2011 Wiley-VCH. (B) Synthesis of the FITC-labeled FA-conjugated CNCs (Sulfo-NHS, N-hydroxysulfosuccinimide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide). Reproduced with permission from ref. 140. Copyright 2014 American Chemical Society. (C) Morphology of the BNC/PNB nanogel: (i) TEM images for the BNC, (ii) PNB nanogel, and (iii) BNC/PNB nanogel P(NIPAm-*co*-BMA), poly(*N*-isopropylacrylamide-*co*-butyl methacrylate). Reproduced with permission from ref. 144. Copyright 2013 American Chemical Society. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

(PNB) and different contents of BNC with a surfactant-free emulsion polymerization [Figure 6(C)]. These nanogel biomaterials showed reversible thermosensitive phase behaviors from a swollen gel to shrunken gel with increasing temperature. At lower temperatures, strong hydrogen bonding between water molecules and the nanogels resulted in fully swollen nanogels; when the temperature increased, the number and strength of H-bonding interactions decreased. This led to the formation of opaque shrunken nanogels. These nanogel complexes may be an ideal candidate for a wide range of medical applications, such as injectable biomaterials and vascular embolization interventional therapies.[144]

Recently, Shi *et al.*[143] reported electroactive hydrogels in which conductive polymers and BNC were combined to build a biology–device interface. In this study, polyaniline or polypyrrole were electrochemically polymerized onto the BNC hydrogel surface; this resulted in a biphasic Janus hydrogel with voltage-responsive properties. Such hydrogels that respond to voltage changes can provide an interface for integrating microelectronics with biology to build implantable devices for future regenerative medicine. More recently, BNC has also been used as a living membrane system for recombinant bacterial strains (e.g., *E. coli*) for potential applications in biological and chemical detection.[142]

## BIOLOGICAL PROPERTIES OF CELLULOSE-BASED BIOMEDICAL MATERIALS: BIOCOMPATIBILITY AND TOXICOLOGY

One of the main requirements of any material for biomedical applications is that it must be biocompatible. Specifically, this is the ability to remain in contact with living tissue without causing any cytotoxic or other side effects. The surface properties of biomaterials, such as the surface charge, chemistry, wettability, topography, and the presence of hydrophobic and hydrophilic domains, all play a vital rule in the cell–biomaterial interactions.[145] In a recent study, Mahmoud *et al.*[146] investigated the effect of the surface charge of the CNCs on the cellular uptake and cytotoxicity. In this study, two differently charged CNCs, CNC–FITC and newly synthesized CNC–rhodamine B isothiocyanate (RBITC), were synthesized [Figure 7(A)]. The *in vitro* cellular uptake studies showed that the positively charged CNC–RBITC was taken up by human embryonic kidney 293 (HEK 293) and *Spodoptera frugiperda* (Sf9) cells without any noticeable cytotoxic effect on the two cell lines, whereas no significant internalization of negatively charged CNC–FITC was observed at physiological pH [Figure 7(B)]. The surface modification of BNC by nitrogen-containing plasma led to a better cell affinity.[145] We found that the plasma-treated BNC improved the cell adhesion and proliferation of the endothelial and neuroblast cells; this was likely due to the improvement in the porosity of the materials.

More recently, the effect of the chemical composition of two different polyanions adsorbed on the multilayer bulk and surface properties and cell response was studied by Aggarwal *et al.*[147] in 2013. In this study, a layer-by-layer technique was used to build films from two different polyanions (Hep and cellulose sulfate) and chitosan as a polycation [Figure 7(C)]. Immune fluorescence studies showed that C2C12 cells plated on terminal cellulose sulfate layers [Figure 7(D)] expressed more longitudinal actin stress fibers and also numerous longer focal adhesions, positive for vinculin, whereas cells were round and loosely attached on chitosan–Hep multilayers. Moreover, during these studies, it was found that the cellulose sulfate-based system showed certain advantages compared to its counterpart (i.e., Hep). For example, it was less dependent on the environmental conditions (e.g., pH) and showed a high bioactivity for the promotion of the adhesion and growth of cells.

Wang *et al.*[148] grafted zwitterionic carboxybetaine brushes from cellulose membranes to improve blood compatibility. The results show that the functionalized cellulose membranes had excellent blood compatibility. The effect of dialdehyde bacterial nanocellulose (DBNC) on the cell adhesion and proliferation was also investigated recently.[149] DBNC can mimic extracellular matrix (ECM) 3D structure and could support the epidermal cell adhesion, proliferation, and proliferation inside the DBNC network. Jia *et al.*[150] studied the biocompatibility of the MCC and CNC composite scaffolds on vascular smooth muscle cell viability, adhesion, and proliferation; this is very important for providing biocompatible scaffolds in tissue engineering applications. We found that the cell viability and morphology within the electrospun scaffolds considerably improved in the case of scaffolds containing both MCC and CNCs compared to those with only MCC or CNCs. This was attributed to the MCC providing anchors for cells to grow within the 3D network of scaffolds and the role of CNCs to improve the cell adhesion. This synergistic effect of MCC and CNCs suggests that cellulose materials are promising additives to potential scaffolds for vascular tissue engineering applications to improve the biocompatibility of scaffolds.[150]

Toxicology studies of cellulose-based materials are still in a very early stage and mainly focus on cytotoxicity. Table I summarizes the most recent toxicology reports for different types of cellulose materials. Overall, there is no evidence of a serious influence or damage of cellulose materials on the cellular and genetic level and *in vivo* animal experiments. However, the inhalation of plentiful cellulose may induce pulmonary inflammation because of the self-aggregation and bioaccumulation of cellulose material in the body.

*In vitro* cytotoxicity studies of CNCs with different cell lines showed no cytotoxic effects at a low concentration range (~50 μg/mL), whereas CNCs induced cell death and changes in the gene expression of mammalian cells at high concentrations (>~100 μg/mL).[151–156] *In vitro* and *in vivo* cytotoxicity and genotoxicity studies of BNC showed no cytotoxicity; also, BNC did not induce any DNA damage, apoptosis, or necrosis in cells under the conditions and concentrations used.[157–159] Finally, the *in vitro* cytotoxicity tests with CNFs and MFC showed no evidence of toxic behavior on the cell membrane and DNA proliferation, whereas chemically modified CNFs showed toxic behavior and negative effects on the cell survival, viability, and proliferation.[160–163] Although several cellulose-based materials have been recognized as nongenotoxic and noncytotoxic, future investigations are needed to comprehensively characterize the toxicology of different types of cellulose materials both *in vitro* and *in vivo*. It is also necessary to assess the potential risks associated with modified cellulose materials because small chemical modification of the material surface could result in drastic behavioral changes in cell–material interactions before they can be broadly exploited.

## CONCLUSIONS AND OUTLOOK

Cellulose materials hold great promise in a wide variety of biotechnological and biomedical applications; these included tissue engineering, drug delivery, cardiovascular applications, wound dressings, and medical implants. This article discusses the

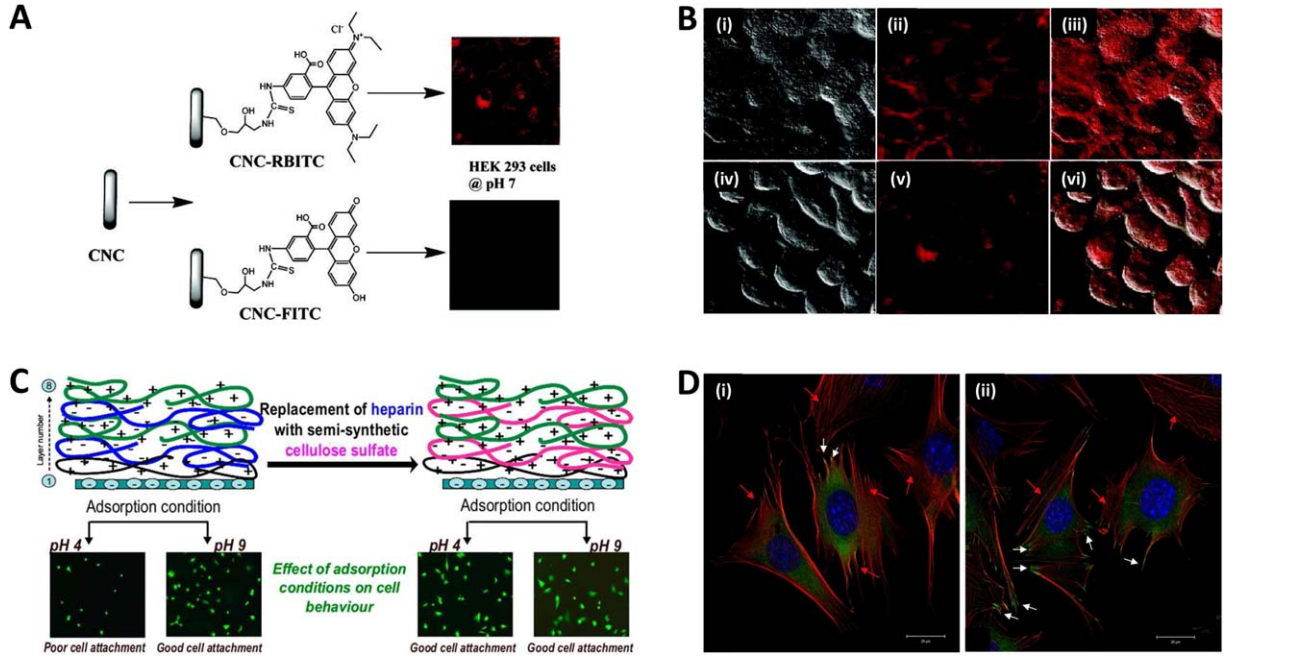


**Figure 7.** (A) Schematic illustration for the synthesis of the CNC–FITC and CNC–RBITC conjugate (B) Confocal images of the Sf9 cells treated with 0.1 mg/mL CNC–RBITC (upper panel) and HEK 293 cells treated with 0.1 mg/mL CNC–RBITC (lower panel): (i, iv) bright field, (ii, v) fluorescent field, and (iii, vi) superimposed fields. Parts A and B reproduced with permission from ref. 146. Copyright 2010 American Chemical Society. (C) Schematic illustration for the layer-by-layer assembly and effect of the adsorption conditions on the cell response. (D) Fluorescence images of the C2C12 cells plated on (i) terminal Hep and (ii) cellulose sulfate layer. The red staining shows actin, the green shows vinculin, and the blue shows the nuclei of cells. The white arrows shows the focal adhesions positive for vinculin. The red arrows show actin stress fibers. Parts C and D reproduced with permission from ref. 147. Copyright 2013 American Chemical Society. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

current state of research on nanocellulose materials in biomedical applications through the discussion of selected most recent works. Undoubtedly, cellulose has great potential for creating a novel class of biomedical materials; this adds both form and functionality. Although in the last decade several successful material-based strategies have been made to advance the field, we believe that in the coming years, there is still potential for significant progress in cellulose biomedical materials. Overall, the creation of reliable and reproducible preparation methods for biocompatible cellulose materials with controlled properties and surface functionalization with tuned form and dispersion within the materials is needed to pave the way for greater advances of cellulose materials in pharmaceutical and biomedical applications.

Among many potential nanocellulose materials, BNC, referred to as microbial cellulose, has been widely investigated and has proven to be a promising candidate in a range of biomedical applications, from topical wound dressings to tissue engineering scaffolds. Preliminary studies on these materials has shown that BNCs are better biomaterials compared to other natural polymers in tissue engineering; this is likely due to the durability and compatibility. In wound-healing and organ replacement applications, several investigations have been made already. This shows their usefulness in these areas; however, much interdisciplinary work is needed to bring BNC materials to commercial products. For instance, a wide variety of mammalian cells need to be cultured onto BNCs to assess their viability and proliferation *in vitro*. Also, a number of *in vivo* studies will be essential to prove its usefulness and functionality for future biomedical applications.

Naturally occurring polymers, such as nanocellulose materials, have been known to be biocompatible and nontoxic for many biomedical applications. However, the chemical functionalization of nanocellulose surfaces with bioactive molecules (e.g., pharmaceuticals, growth factors, anticoagulants or coagulation cascade factors, and angiogenic factors) for improved interactions with the human body is an important area with tremendous potential for paving the way in specific biomedical applications. The surface chemistry, architecture, and structure of cellulose materials significantly influence the cellular adhesion, proliferation, and differentiation. Hand in hand with surface functionalization, the form of cellulose used for biomedical applications (e.g., hydrogel, solid film, NC) provides a useful and powerful tool to tune the interactions of biomaterials with living tissue. Although nanocellulose materials are not inherently dangerous, more *in vitro* and *in vivo* studies are needed to evaluate the potential pharmaceutical side effects and cytotoxicity of this promising natural biopolymer.

Clearly, despite the significant developments concerning biomedical nanocellulose-based materials, this area is still in in its infancy. We believe that there are still several areas that need to be addressed and plenty of possibilities to be explored in this topic. As materials science continues to rapidly develop in

**Table I.** Examples of the Toxicological Evaluation of Cellulose-Based Materials for Biomedical Applications

| Cellulose type | Toxicological experiment | Results | Reference |
|---|---|---|---|
| CNCs | *In vitro* cytotoxicity test of CNCs isolated from cotton with a 3D triple-cell coculture model of the human epithelial airway barrier | Lower cytotoxicity and (pro)inflammatory response in comparison with multiwalled carbon nanotubes and crocidolite asbestos fibers | 151 |
| | *In vitro* cytotoxicity test of CNCs extracted from cotton with a flow cytometry assay | The low concentrations (0.02–100 μg/mL) of CNCs did not show cell death. However, high concentrations (>200 μg/mL) induced cell death and changes in the gene expression of mammalian fibroblasts. The high concentrations (2000 and 5000 μg/mL) of the CNCs affected the expression of stress- and apoptosis-associated molecular markers. | 152 |
| | *In vitro* cytotoxicity of the CNCs isolated from cotton with an thiazolyl blue tetrazolium bromide (MTT) assay with 3T3 fibroblast cells | CNCs within the concentration range of 100–1000 μg/mL induced minimal decreases in cell viability after 1 day of cell exposure. | 153 |
| | *In vitro* cytotoxicity evaluation of CNCs with nine different cell lines | No cytotoxic effects in the concentration range (0–50 μg/mL) and with an exposure time of 48 h | 154 |
| | *In vitro* cytotoxicity evaluation of CNCs with L929 cells | Low cytotoxicity of CNCs at low concentrations | 155 |
| BNC | *In vitro* genotoxicity of BNC nanofibers: (1) *Salmonella* reversion assay, (2) proliferation assay with mouse embryo fibroblasts (3T3) and Chinese hamster ovary cells, and (3) single-cell gel assay (comet assay) | No mutagenic behavior under conditions used, 10–20% lower proliferation rate in the presence of BNC nanofibers, and BNC nanofibers did not induce DNA damage under the concentrations tested | 157 |
| | *In vitro* and *in vivo* cytotoxicity of BNC in human umbilical vein endothelial cells (with viability and flow cytometric assays) and mouse model | No toxicity in endothelial cells<br>No biochemical differences were observed after 7 days in animal experiments. | 158 |
| | *In vitro* and *in vivo* toxicity of BNC in human umbilical vein endothelial cells and mouse model | BNC did not induce apoptosis and necrosis in endothelial cell and did not stimulate an immune response in endothelial cells and a mouse model. | 159 |
| CNFs | *In vitro* cytotoxicity test of neat CNFs and modified CNFs with fibroblast 3T3 cells | The neat CNFs did not exert toxic behavior on fibroblast cells. The neat CNFs showed no effect on the cell membrane, mitochondrial activity, or DNA proliferation. The modified CNFs showed toxic behavior and negative effects on cell survival, viability, and proliferation. | 160 |
| | *In vitro* genotoxicity of CNFs with enzyme comet assay | No significant DNA damage | 161 |
| MFC | *In vitro* toxicity of unmodified cationic and anionic MFC with human dermal fibroblasts | No cytotoxicity of MFC was observed independently of the chemical treatments. | 162 |
| | *In vitro* cytotoxicity test of MFC with mouse macrophages and human monocyte-derived macrophages | No evidence of inflammatory effects or cytotoxicity on mouse and human macrophages was observed after 6 and 24 h of exposure to the materials studied. | 163 |

biomedical fields, nanocellulose materials may provide a solution in the future for overcoming some of the insurmountable challenges of biomedical materials.

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