Promising Eco-Friendly Biomaterials for Future Biomedicine: Cleaner Production and Applications of Nanocellulose

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Abstract

Cellulose nanomaterials are developing nowadays as a promising environmental friendlier biomaterial that can be extracted from native plant sources. These materials, because of their excellent physico-chemical and biological properties, urged much interest in biomedical applications. In this paper a detailed review on nanocellulose is provided with special emphasis on greener production, demands, bio-properties and innovative application in some selected biomedical fields such as tissue implants, drug delivery systems, wound healing and biosensor applications. The current state of global commercial nanocellulose based product and its future potentials for biomedical applications are also discussed.

1. Introduction

Biomedical devices and implants constitute a major share in the field of biomedicine, where a wide variety of synthetic materials are utilized nowadays. However, because of the numerous side effects of these materials, researchers have directed their efforts at the invention of more advanced and non-toxic nanomaterials (Subhedar et al. 2020). The manufacturers and researchers have, at present, concentrating on renewable resources. Cellulosic nanomaterials can fulfil almost all the criteria for being “green” because of its naturally derived sources and biodegradability at the end of its life cycle (Reshmy et al. 2020a). In general, 'green' refers to materials, processes, and products that have fewer impacts on the environment and are less detrimental to human health than conventional ones. Nanocellulose (NC) has gained significant attention as eco-friendly, versatile and excellent biocompatible material (Dufresne 2013; Lin and Dufresne 2014a; Ahmad et al. 2018). It is tremendously advocated by researchers' interested in state-of-the-art green techniques for many biomedical applications because of the outstanding properties of NC. The composite materials based on NC exhibit excellent chemical, biological and physical properties along with environmental capabilities, which make them ideal nanomaterials for long-term practical application (Jonoobi et al. 2015). NC can be synthesized from different agricultural wastes as well as waste paper. In the purification stage, waste paper requires lower quantities of chemicals than from lignocellulosic raw materials, since it has also gone through phases of different chemical treatments. It is the most cost-effective and eco-friendly strategy because it also helps to manage waste (Usov et al. 2015). The benefit of using biomaterials over others is that they enable the material to decompose gradually, followed by tissue regeneration. These NC-developed biomaterials have been used in tissue engineering scaffolds, biomedical implants such as blood vessel growth, breast prostheses, urethral catheters, adhesion, artificial skin, articular cartilage repair, barrier, drug release systems, regeneration of dura-mater and gum, diagnostics and testing, etc (Torriani et al. 1990; Feijo et al. 2005; Negro et al. 2006; Bhat et al. 2018).

Numerous NC-based materials have been developing everyday and are also being revealed for variety of biomedical applications. Tissue engineering is an emerging field and possesses innovative potential for NC biomaterial for developing biomedical replacements. Recent researches have drastically enhanced knowledge of the significance of 2D and 3D culture systems in cell activity. 3D cell cultivation offers a more physiological conditions environment for directing cell behaviours and enhancing their

functions (Kittur et al. 1998; Puschmann et al. 2013; Thoma et al. 2014; Ma et al. 2015). Therefore, in order to replicate the biological and micro-environment components, immense efforts have already been reported about the development of 3D biofabrication biomaterial systems forming complex functional 3D structures with suitable cell types. Biomedical devices like biosensors are also crucial for early diagnosis of diseases. Different biosensors based on NC have been developed in recent years for different tissue engineering and regenerative medicine applications. It can monitor real-time biological signals such as proteins response to damaged tissue, cardiac infarction, inflammatory reactions, release of antibodies, muscular dystrophy (Sharma and Bhardwaj 2019). Because of the high-modulus of nanocellulose, it can be revealed as a promising nanoreinforcement for biomedical devices with homogeneous dispersion and good interfacial adhesion, enabling adequate stress transfer from matrix to the reinforced materials (Xue et al 2017; Sharma and Bhardwaj 2019; Subhedar et al. 2020). The issues related to the practical hurdles for designing and developing of biomedical devices based on NC are also crucial to discuss for implementing NC based commercial products. With these concerns, the present review discussed NC-based biomaterials with special emphasis on their cleaner production, properties, commercial scope, challenges and diverse applications in the biomedical field.

2. Cleaner Waste Conversion Strategies Of Nanocellulose Production

A variety of cellulose-based materials are included in the term NC, where physical, chemical and biological properties usually differ depending on their source and method of extraction. There are mainly three types of NCs namely cellulose nanofiber (CNF), cellulose nanocrystal (CNC) and bacterial nanocellulose (BNC) are to be isolated from different sources (Thomas et al. 2019). The CNF has approximately $>1\mu\text{m}$ length and is flexible, consists of compressed connections of cellulose fibres in the plant (Dufresne 2013). By combining chemical, enzymatic treatment and mechanical pressure delamination of the pulped form of wood/plant can be utilized as a tool for isolating CNF. CNC is a crystalline structure that is rod-shaped, elongated and less flexible (Lu and Hsieh 2012). Acid hydrolysis produces CNC with considerably low aspect ratios of 2-20 nm crystal diameter (Mascheroni et al. 2016; Wulandari et al. 2016). BNC has a diameter of less than 100 nm, with twisted ribbon fibrils consisting of fine 2-4 nm nanofibrils (Castro et al. 2011). BNCs have proven to have exclusive mechanical, physical and structural properties in many areas of biomedical applications to become an economically useful material (Sani and Dahman 2010; Shezad et al. 2010; Gitari et al. 2019).

Many types of extraction routes are already reported for nanocellulose production. These include mechanical, chemical and enzymatic treatments for defibrillating microfibrils of cellulose. Usually, chemical methods such as alkaline treatment (Alemdar and Sain 2008; Deepa et al. 2015) and acid hydrolysis (Elazzouzi-hafraoui et al. 2008; Reshmy et al. 2020b) are used, while enzymatic hydrolysis (Henriksson et al. 2008) is often used for nanofibril production. The mechanical treatments include grinding (Abe and Yano 2010), cryo-crushing (Wang and Sain 2007), high pressure homogenization (Norio and Hiroyuki 2008) etc.. To make the process more environmental friendly some researchers reported the extraction of nanocellulose using novel methodologies. For such initiatives, Reshmy et al., reported bleaching of raw materials using natural agents like soapnut (Reshmy et al. 2021), Lu et al.

extracted CNC using a green catalytic route by put forward application of phosphotungstic acid under mechano-chemical activation for the hydrolysis of cellulose raw materials (Lu et al. 2016), and Miao et al developed a one pot synthesis for the production of hydrophobic CNC from hardwood pulp using tetrabutylammonium acetate/dimethylacetamide system (Miao et al. 2016). In several cases a combination of reactions is necessary to achieve complete isolation depends on the extent of purity and dimensions required for specific applications. Deep eutectic solvent pretreatments are also reported to be environment friendlier assuring the manufacture of NC with a microwave-supported pretreatment followed by high-intensity ultrasonic treatments (Liu et al. 2017b). Ammonium persulphate has both delignification and oxidative degradation capacity that can directly implement without pretreatments of the raw materials for the production of CNC (Cheng et al. 2014). American Value Added Pulping technique is an effective low-cost method for commercial production of NC from various types of lignocellulosic wastes (Nelson et al. 2016).

The applications of NC for different fields are catching the attention of technologist for designing non-toxic and eco-friendly implants for biomedical uses. The applications of NC as a polymer nanocomposite with a number of polymers, hydrogels and biomaterials are of advanced potentials to design and develop devices for specific applications (Naz et al. 2019).

3. Demand Of Nanocellulose For Biomedical Applications

NC has numerous characteristics distinct from conventional materials as a natural nanoscaled material, including crystallinity, nontoxic, specific morphology and geometric measurements, huge surface area, biodegradability, rheological characteristics, liquid crystalline nature, orientation and alignment, biocompatibility, high mechanical strength, barrier properties and pronounced surface modification possibilities. Characteristics of the three main types of NCs are listed in Table 1. Nano-enabled NC biocomposites have been envisaged on the basis of these specific properties, varying from bulk applications such as composite reinforcement and rheological modifier in biomedical fields namely drug delivery, wound healing, tissue engineering, and biosensors (Lin and Dufresne 2014b).

NC has been widely used as a filling material in biomedical composites due to its high mechanical strength, biodegradable, biocompatible and hydrophilic nature (Septevani et al. 2017). A pictorial representation of different characteristics of NCs as a biomedical component is shown in Fig.1. While also comparing the existence of proteins, it prevents antagonistic tissue responses; its composition decreases hemolytic and immunogenic responses. In addition, it promotes tissue interaction as well as growth. NC degrades very gradually in both in-vivo and in-vitro settings, which makes it perfect for long-term applications. It is extremely wear resistant and can withstand heavy materials, so it can be used for long-term applications as a scaffold (Lina et al. 2011; Subhedar et al. 2020). Biomedical products include dermal treatments for burnings and cuts; procedure of drug distribution; development of soft tissue; scaffolds; implants; as well as formation of hard tissue. In influencing cellular adhesion, relocation, propagation, and functions, its aspect decrease tissue reactions, while growth factors stimulate tissue regeneration (Dugan et al. 2013; Pandey et al. 2015). They also help tissues, provide a

medium for fresh cells, and maintain the necessary environment for development. BIOFILL was the first commercially available use of BNC for skin tissue repair in 1990 and as artificial blood vessel for microsurgery in 2001 (Sharma and Bhardwaj 2019). NC, which is valuable in biosensors and diagnostics, serves as an effective medium to immobilize biomolecules. For example, a single-step biotemplate technique was used to synthesize gold BNC composite in water suspensions. This nanocomposite demonstrates remarkable conductivity as well as biocompatibility. NC are often used for antibody immobilization by initially carboxylated and further activated by coupling with two proteins. NC layer supports different functions such as feasible drug application, soaks up discharge pus, has a sedative effect, prevents inflammation and fluid loss and allows NC physicochemical characteristics suitable for the repair of skin tissue (Pereira et al. 2013a). Usually, wound healing materials are absorbent and cling to the wound surfaces, involving a lot of trauma and pain in subsequent removal during wound recovery (Czaja et al. 2006). BNC is a strong wound dressing agent as it regulates the pus discharge and maintains a damp environment for better wound recovery (Poonam and Chauhan 2020). Hydrogels based on CNCs and CNFs show most of the properties needed for the repair of skin tissue, which are common due to simple processing and tunable mechanical characteristics (Du et al. 2019). Materials that are to be used as a biomedical implant are essentially biocompatible, as it replaces, physically and chemically suits the tissue features. In biomedical implants, NC is a good material to be used as it is not weakened in the body environment and offers resilient chemical and mechanical stability. NC is a suitable fit for ear cartilage implants, since it can be tailored according to the patient's ear shape (Nimeskern et al. 2013). NC is also used for bone cartilage replacement (Amorim and Costa 2009). In dental applications, NC plays a special role in protecting the artificial filling implemented to the tooth and also stimulates the rapid regeneration of the alveolar bone. These NC membranes look like the original tissue after 50 days. Properties such as biocompatibility; bioactivity and osteoconductivity make NC a good bone replacement material. Often, NC and hydroxyapatite composite is used as a bone regeneration scaffold (Poonam and Chauhan 2020). Due to the aforementioned characteristics of NC, these materials and their composites have promising acceptability as a candidate for potential biomedical applications.

4. Bio-properties: Toxicology And Biocompatibility

One of the foremost necessities of any biomaterial for application in medicine is that it should be compatible with cells or tissue. This is precisely the capacity to continue to communicate with living cells or tissue thereby having no toxic effects. The biomaterial characteristics, like charge of surface, chemistry, topography, wettability and the existence of various domains which hydrophilic or hydrophobic, all exhibit a vital role in the biomaterial-cell interactions. DeLoid et al conducted toxicological analysis of consumed NC in both *in vivo* and *in vitro* systems. The *in vitro* experimental in a standardized food model based diet in American rats showed a gastrointestinal tract simulation due to the two forms of NC namely CNC and CNF at 0.75 or 1.5% w/w.. No noteworthy changes in serum markers, hematology, or histopathology were perceived between control groups and rats fed with CNF suspensions. These experimental results propose that ingested NC has no toxicity, and is probably non-hazardous when ingested in slight amounts (DeLoid et al. 2019).

Similarly, in another study, CNC was experimentally digested using a three-phase digestion simulator (oral-gastric-small intestinal) and subsequent digestion was applied to the human cellular model of the small intestinal epithelium in vitro triculture from the small intestinal phase (SIE). Exposure effect on cytotoxicity, oxidative stress and SIE monolayer integrity, were assessed, and a proteomic analysis was conducted to obtain complete knowledge of the cell responses to consumed CNC in diverse models of food. In the presence 1.5% w/w CNC only slight cytotoxicity was reported and interestingly CNC did not considerably affect SIE health(Cao et al. 2020). The effect of the surface charge of the NC on cytotoxicity and cellular uptake was explored by Mahmoud et al. Two differently charged CNCs, CNC-rhodamine B isothiocyanate and CNC-FITC, have been synthesised in this work. The in vitro cellular uptake experiments showed that HEK 293 cells took up the positively charged CNC-rhodamine B isothiocyanate without any apparent cytotoxic impact on these cell lines, although no notable CNC-FITC (negative charged) internalisation was observed at physiological pH (Mahmoud et al. 2010). The modification of surface of NC (bacterial) by plasma which contains nitrogen led to a better affinity to cell (Pertile et al. 2010). Cytotoxicity analysis of cellulose-based biomaterials are still at its infancy stage. Table 1 outline the latest toxicology analysis for the three different forms of NCs. Generally, there is no evidence that cellulosic biomaterials have a significant effect or damage on genetic, cellular and in vivo animal models.

5. Applications In Biomedical Field

The fabrication of novel NC based biomaterials for biomedical applications is always an interdisciplinary topic of research. In 2012, the Future Markets Inc. forecasted a growth rate of approximately \$97 billion for NC-impacted medical and life sciences markets by the year 2017 (Sheikhi 2019). The basic characteristics of an ideal biomaterial correlate with its high mechanical strength, structural diversity, biocompatibility, cell adhesion, hydrophilicity, ability to facilitate cellular interactions, proliferation, porosity and chemical composition (Shatkin et al. 2014; Rajwade et al. 2015). While degrading the basic infrastructure and properties, NC, in particular BNC, can be sterilized and changed to a suitable implantable biomaterial providing a variety of unique biomedical applications (Naz et al. 2019). Applications of NC in different biomedical fields are depicted in Fig.2. The purpose of this section is to address the biomedical application of NC in various fields such as tissue engineering and implants, drug delivery systems, wound healing and biosensor applications.

5.1. Tissue engineering and tissue implants

There have been many promising researches based on advancement of NC in bone, cartilage, neural, liver and vascular tissue engineering (Bacakova et al. 2019). NC possesses very high potential for bone tissue engineering. NC/chitin composite scaffolds were proved for stimulating the adhesion and multilayered growth of bone marrow mesenchymal stem cells (MSCs) that is derived from rat femur (Torres-Rendon et al. 2015; Vielreicher et al. 2018). The modifications of NC using biomimetic mineralization with hydroxyapatite or tricalcium phosphate were reported to improve the performance of MSCs and other bone-forming cells (Sundberg et al. 2015; Si et al. 2016; Valentim et al. 2018). NC based scaffolds coupled with collagen were also designed for replacing bone tissues (Saska et al. 2017). A hybrid

composite of CNCs, bioactive glass (45S5) and stainless steel (316L) is used for strengthening bone healing process (Chen et al. 2015). Rashad et al constructed a 3D-printed NC/polycaprolactone scaffolds to augment the regeneration of human bone marrow-derived mesenchymal stem cells (Rashad et al. 2018). NC microporous scaffolds provided growth of smooth muscle cells, while nanoporous ones supported epithelial cells (Lv et al. 2018).

BNC has been extensively applied in cartilage tissue engineering as auricular cartilage scaffolds due to its host tissue response, high water-retention capacity and mechanical strength. Bilayered BNC/alginate scaffolds were also reported for the development of human nasoseptal chondrocytes because of their non-pyrogenic and non-cytotoxic nature (Martínez Ávila et al. 2015). Laser modified BNC scaffolds have been used for articular cartilage engineering as substrates for the growth of human chondrocytes and found better diffusion of nutrients for the growth and differentiation of chondrocytes (Ahrem et al. 2014). The application of NC based bioink in 3D bioprinting of anatomically formed cartilage structures, such as sheep meniscus and human ear was another novelty (Markstedt et al. 2015). BNC/alginate sulphate bioinks are also reported for developing bovine cartilage that facilitated the spreading, proliferation, and collagen synthesis (Muller et al. 2017). Pereira et al developed a CNC/ Gellan gum hydrogels for regenerating the intervertebral disc (Pereira et al. 2017).

In neural tissue engineering, BNC is utilized to cultivate SH-SY5Y neuroblastoma cells and its functional action potentials were identified by electrophysiological recordings (Innala et al. 2014). The 3D BNC scaffolds were developed by cationic modification using trimethyl ammonium betahydroxypropyl derivative of cellulose to enhance adhesion, proliferation and development of neuronal 3D networks on PC12 cells (Brackmann et al. 2015). NC based scaffolds were also reported as innovative tools for brain neural tissue-engineering. In 3D printing of electrically conductive scaffolds, an ink containing carbon nanotubes together with wood-derived CNFs was used, which facilitated the adhesion, proliferation and differentiation of human SH-SY5Y neuroblastoma cells (Kuzmenko et al. 2018).

The first concept was to construct a 3D culture of hepatic cells in liver tissue engineering, which is more biocompatible than the 2D culture commonly used to predict and assess the excretion, toxicity and metabolism in the human liver. Malinen et al developed a 3D scaffolds based on birch wood-derived CNF for regenerating several human liver cell (Malinen et al. 2014). In vitro models of BNC/alginate 3D scaffolds were constructed in adipose tissue engineering for the treatment of metabolic diseases (Krontiras et al. 2014). Similar types of scaffolds with high viability and effective mature phenotype were synthesis by cross linking NC, hyaluronic acid and adipocytes for adipose tissue engineering (Henriksson et al. 2017). Using a plant-derived CNF hydrogel, a versatile 3D environment for hPSC culture, imitating the 3D in vivo stem cell niche, was developed and that preserved the pluripotency of hPSCs for 26 days (Pluripotent et al. 2014).

Tubular structures from BNC have been developed for vascular tissue engineering using silicone tubes for replacing hollow organs such as urethra and oesophagus (Hong et al. 2015). Weber et al. reported an in vivo study proving the biocompatibility of BNC tubes to substitute the right carotid artery in sheep (Weber

et al. 2011). Another fascinating concept was to use super paramagnetic iron oxide nanoparticles (NPs) coupled with BNC to coat endovascular stents, which would then compel vascular smooth muscle cells (VSMCs) to rebuild the tunica media in blood vessels in situ (Echeverry-rendon et al. 2017). Magnetic BNC coated with polyethylene glycol has been shown to shape sufficient scaffolds for porcine VSMCs in vitro experiments, showing minimum cytotoxicity and supportive effects on cell migration and viability (Bacakova et al. 2019). BNC scaffolds were also reported as 3D platform for monitoring efficiency of antitumor drugs (Reis et al. 2017). In future, different forms of NCs can be designed for potential tissue engineering and implant developments.

5.2. Drug delivery

Recent progresses in nano biomaterials have created a number of diverse systems for drug carrier applications. NC based products have already acquired approval by United States Food and Drug Administration and have been used for drug delivery because of their essential characteristics such as biocompatibility, morphology and geometrical dimensions (Salas et al. 2014; Abitbol et al. 2016; Shivaramakrishnan et al. 2017). Based on its chemical modifications, potential multifunctionalities may be utilized to bind and release different therapeutic agents (Kupnik et al. 2020). NC has become increasingly appealing over the last decade because of its ability to control the release time depending on its functionality (Kolakovic et al. 2012; Lin and Dufresne 2014b). For example, cellulose acetate (CA) is implemented in formulations of a pain reliever and two antibiotics in HIV medications in which CA has different drug release rate. In oral drug delivery formulations also NC namely hydroxypropyl methylcellulose has been used. One of the main aims of utilizing NC as drug recipients is to monitor the rate of drug release and attain the proper concentrations of drugs (Lin and Dufresne 2014a). In addition, cellulose derivatives can move safely through the human digestive system, and can be broken down in the gastrointestinal tract into natural metabolites by digestive enzymes (Plackett et al. 2014).

Trovatti et al. reported BNC membranes as topical release systems of lidocaine in which over 90% of the total drug was released during the first 20 minutes itself (Trovatti et al. 2011). In another study, Muller et al. reported the mechanism of BNC as a possible drug delivery system for serum albumin proteins and illustrated that during the process, the biological stability of albumin was preserved (Muller et al. 2013). Abba et al. investigated the direct dissolution and transdermal passage after incorporation of crocin into BNC membrane (Abba et al. 2019). The idea of drug delivery systems based on NC for amine-containing drugs was reported by Dash and Ragauskas (Dash and Ragauskas 2012). Chang and Wang has recently developed NC based system in which hydrogen peroxide and oxygen released during process that modulate the growth of mammalian cells (Chang and Wang 2013). Another attractive cellulose substrate namely carboxymethylcellulose (CMC) is also attracted growing interest in the drug delivery sector in recent years (Weng et al. 2013; Roy et al. 2014). For improved cytotoxicity against cancer cells, Ernsting et al. developed CMC-based systems loaded with docetaxel (DTX) (Jorfi and Foster 2015). Wen and Oh were identified a dual-stimuli-responsive system with CMC nanogels as a possible intracellular anticancer drug delivery carriers (Wen and Oh 2013).

In order to determine the suitability of NC as multifunctional NPs that can be used for simultaneous in vivo imaging and cancer treatment, numerous studies have been performed. Multiplex NPs may be used to identify malignant cells and these NC based systems tracking progress in real time, destroying most cancer cells with limited side effects (Tan et al. 2019). The linking of curcumin on NC based materials has been investigated as an effective system. Zainuddin et al. isolated and modified CNCs from the kenaf bast fibers with the cationic surfactant cetyltrimethylammonium bromide and developed drug delivery systems with curcumin (Zainuddin et al. 2017). The use of CNC films for the delivery of antimicrobial drugs in diabetic wound dressing was studied (Yenn et al. 2017). Li et al. developed CNCs with folate, cis-acetonitrile-doxorubicin, and polyethyleneimine.. The hybrids released 95% of doxorubicin at pH 5.5 at 24 h (Li et al. 2019). Hivechi et al. synthesized and analyzed CNC-based system with poly(ϵ -caprolactone) for its drug release activity (Hivechi et al. 2019).

Löbmann and Svagan reported CNFs in drug formulations, with a emphasis on poorly soluble drugs in which CNFs are used to achieve long-lasting continuous release as stabilizers and act as film and foam formers (Löbmann and Svagan 2017). CNF aerogels were studied by Bhandari et al. as carriers for orally controlled drug delivery systems (Bhandari et al. 2017). These aerogels displayed positive floatability and mucoadhesive properties. Paukkonen et al. reported an emulsion for immediate and sustained drug release applications (Paukkonen et al. 2011).

5.3 Wound healing and antibacterial properties

NC based materials and hydrogels have extensive clinical uses in wound healing as medicinal materials. In order to diminish the possibility of scar formation while facilitating epithelization and cell migration into the wound, these wound dressings should provide a wet environment (Sun et al. 2014). In order to withstand external threats such as bacteria, foreign bodies, or tissue-damaging factors, these wound dressings should also have high mechanical strength and flexibility (Koehler et al. 2017; Zhao et al. 2020). NC-based hydrogels fulfil most of the criteria and have therefore recently been used for the application of wound dressing (Liu et al. 2016; Sun et al. 2017; Huang et al. 2018). The NC hydrogels also mimic the biological tissue particularly because of its porous nature (Liu et al. 2017a; Subhedar et al. 2020).

Wound infections caused by high levels of bacteria, particularly traumatic injuries, burn wounds and surgical procedures, are an important reason for delayed or prolonged wound healing. In the fabrication of biomaterials, NC may offer a porous network like orientation that is useful for the possible transfer of antibiotics or suitable medicinal products into the wound, thus acting as an important physical barrier against any external infection (Andresen et al. 2007). Silver has been studied and used most extensively since ancient times among the various antimicrobial agents to fight infections and prevent spoilage. In order to introduce Ag NPs as antimicrobial agent into NC-based products, chemical reduction and simple impregnation are popular methods. Currently, a number of studies have endeavoured to integrate both Ag NPs and CNC into polymeric matrices in order to provide synergistic effects of mechanical reinforcement and antibacterial agents such as PLA/Ag/CNC (Fortunati et al. 2012, 2014), poly(3-hydroxybutyrate-3-

hydroxyvalerate)/CNC/Ag (Yu et al. 2014) and water borne polyurethane/Ag/ CNC (Liu et al. 2012). The antimicrobial efficiency of Ag NPs relates to the size and shape of NPs in NC based biomaterials. There have been claims that CNC nanohybrid materials containing dendritic nanostructured Ag demonstrate stronger antibacterial activity than sphere nanostructured Ag (Xiong et al. 2013). As far as antibacterial biomaterials from CNF and Ag NPs are concerned, it has been reported that composites composed of CNF and Ag NPs can be generated by an electrostatic assembly approach via polyelectrolytes as macromolecular linkers between CNF and Ag NPs (Martins et al. 2012). Liu et al. investigated the incorporation of chitosan-benzalkonium chloride or chitosan-methylisothiazolinone as antibacterial mediators to sodium alginate/CNF antibacterial composites and found that these composites shows potential mechanical strength and exceptional antibacterial action against *Staphylococcus aureus* (Liu et al. 2013)(Liu et al. 2014). The gentamicin-RGDC-grafted BNC membranes have been reported to be bactericidal against *Streptococcus mutans* but non-toxic to human dermal fibroblasts, showing possible use in drug delivery systems and wound healing (Rouabhia et al. 2014).

Recent advances in antibacterial property of NC are by surface modifications, which imply the production of NC antibacterial biomaterials without using an antibacterial agent. For example, the CNF surface modification can be achieved by using amino-functionalized through a nucleophilic displacement response starting from cellulose-p-toluenesulfonic acid ester. Interestingly, high antimicrobial action against *Staphylococcus aureus* and *Klebsiella pneumonia* was documented in electrospun polyvinyl alcohol (PVA) nanofibers containing this amino-modified CNF(Roemhild et al. 2013). The chemical grafting of aminoalkyl groups on the surface of the BNC nanofibrillar network to provide its antimicrobial activity was also recorded in a similar study (Fernandes et al. 2013). The use of partly deacetylated chitin nanocrystals in BNC materials to produce all-polysaccharide antimicrobial composites was documented by Butchosa et al. With 99 ± 1 per cent bacterial growth inhibition, this green composite with all natural components was stated to have shown good antibacterial activity. (Butchosa et al. 2013). BNC composites integrated into small amounts of Cu-montmorillonite, but without any investigative mechanism, have shown some antimicrobial activity. Most reported surface modified NC materials have potential antimicrobial effects against Gram-positive and Gram-negative bacteria. However, several interesting characteristics and key problems on antimicrobial biomaterials based on NC are still unidentified, particularly with regard to the fair balance between antimicrobial activity improvement, antimicrobial effect period and the regulation of normal human cell destruction (Lin and Dufresne 2014a). Fan et al. developed an in situ cross linked hydrogels formed by the cross linking of oxidized CMC and carboxymethyl chitosan for healing second-degree post wounds in 14 days within rat model without any major adverse reactions (Fan et al. 2012).

5.4 Biosensors

NC, which is valuable in biosensors and diagnostics, serves as an effective material for immobilizing biomolecules. For example, a single-step biotemplate technique has synthesized gold BNC composite in water suspensions. This nanocomposite demonstrates remarkable conductivity as well as biocompatibility. Its thin nanofiber organization is capable of trapping and conserving its biological

activity with an enzyme called horseradish peroxidase (HRP). The HRP enzyme biosensors are capable of detecting H_2O_2 at a concentration below 1 μM (Zhang et al. 2013). NC layers, which are often used for antibody immobilization, are initially carboxylated and further activated by coupling with two proteins. This activated layer can, by physical adsorption; trap antibodies called antihuman immunoglobulin G. Copolymer grafting can also activate the NC surface. To improve the selectivity of the NC sheet, the grafted polymer is conjugated with peptides with a strong affinity for human antibodies. They are immobilized with certain proteins or growth factors to improve NC's biocompatibility. Adhesion is strengthened by the use of some sequences of amino acids (Andrade et al. 2009). The chitosan polymer can be used as a spacer that is adsorbed on its surface to further improve the specificity and binding potential of NC (Yang et al. 2006). In another study, DNA has been embedded into cotton-derived NC/AgNPs to prevent agglomeration in much sodium borohydride as a reducing agent (Liu et al. 2011).

Dong et al developed NC embedded folic acid and made them fly to the folate receptor of rat and human brain cell tumor cells. In vitro analysis demonstrated that the cellular binding of fluorescein isothiocyanate by the folate receptor to the NC embedded folic acid was much greater than that of folic acid alone (Dong et al. 2014). Zhai et al investigated a very sensitive and reversible NC- based Platinum nanoparticles-PANI nanohydrogel for glucose biosensing (Zhai et al. 2013). Li et al. developed a low-cost, scalable and flexible hierarchical nanohydrogel-based biosensor platform, which can be developed for responsive and quick detection of human metabolites such as cholesterol, uric acid and triglycerides (Li et al. 2014). NCs are excellent substrates and/or carriers with exceptional electrical, optical and mechanical properties for the fixation of different types of NPs (Hamedi et al. 2014; Golmohammadi et al. 2017). NC can also be used as biosensors in different sensor applications to display analytical information, such as food safety, clinical/medical diagnostics, physical sensing, environmental monitoring, and labelling, and bioimaging applications.

6. Commercial Status Of Nanocellulose Production

Companies including Future Markets Inc., Allied Market Analysis, Global Industry Analysts Inc., and Market Intel, LLC have predicted the boom of the nanocellulose market in the near future in a variety of studies and end-user guides (Ferreira et al. 2020). The unifying themes were the need to quest for high value added applications and recognise the cost proposition in terms of light-weight and low loading requirements for replacing other materials. About 90% contributions to commercial developments in NC-based innovations are reported by countries such as United States, Japan, China and Brazil (Charreau et al. 2020).

There are various and varied application possibilities, from large scale manufactured products for high-end medicinal commodities. All the three forms of NC may be utilized in some fields, while the form is more important for other applications. BNC, for example, is especially suitable for applying as medicinal implants, cell culture, wound healing and drug delivery scaffolds (Klemm et al. 2018). The application of BNC to substitute scratched skin temporarily tiled the way for availability of products based on NC for wound healing applications were reported (Weber et al. 2011; Gama and Dourado 2018). The commercial

applications of NC based products in different biomedical fields are depicted in Table 3. For various forms of wounds, such as skin graft donor sites, pressure sores, venous stasis, skin tears, diabetic wounds and traumatic abrasions, this collection of commercial products is feasible (Sharma and Bhardwaj 2019; Kupnik et al. 2020). Double layered membrane developed from alkali-modified BNC and pristine was reported for application as a dental biomaterial that decreases inflammatory responses to treat periodontal diseases by guided tissue regeneration (GTR) (Ferreira et al. 2020). While NC scaffolds derived from plants have the capability to meet the needs of tissue engineering, there is yet to be a commercial market breakthrough. For instance, one of the only commercialised drugs is Growdex (UPM Biomedicals, Finland), a CNF-based product designed to promote cell growth and differentiation. The number of scientific studies showing promising in vivo behaviour of NC scaffolds for particular tissue engineering applications is rising encouragingly (Ma et al. 2015; Kuzmenko et al. 2018). However, for a clear understanding of NCs as applied to tissue growth synchrony, which is an intricate phenomenon, crucially related to various environmental signals (Salgado et al. 2004; Nasiri et al. 2018). Moreover, the concern of medical trials in humans is another remaining obstacle. Zhang et al reported BNC membranes for GTR and restore maxillary canine periodontal defects in beagle dog breeds (Zhang et al. 2018). When utilized as a replacement for dura mater in mongrel dogs, BNC also demonstrated strong approval and compatibility to bone graft fragments. In a multicenter clinical trial in the USA, effective in vivo tissue engineering functions in human regenerative medicine have also been reported (Rosen et al. 2011).

The potential implementation of CNF and CNC biocomposites as scaffolds in biomedical fields strongly supports this positive development and efficient performance of NC biomaterials (Du et al. 2019). There is some need to follow the regulatory permission for production trials needing specific facilities. This is the major hurdle for academic researchers to perform the transformation from laboratory technology to the clinical trials (Roseti et al. 2017). However, any such transformation could ultimately generate economic stimulus, as the sales of tissue engineering biocomposites are already above US\$ 240 million per year and about 25% of the US GDP is projected to be linked to healthcare by 2040 (Place et al. 2009). Therefore, it may be interesting to bridge the difference between research and commercialization.

7. Future Prospects And Conclusions

In order to optimize the methodologies to adapt the characteristics and properties of NC, future research requires primarily sustainable, eco-friendlier and green extraction techniques, such as mechanical extraction and enzymatic hydrolysis. Most of the technologically advanced studies have been conducted successfully on a laboratory level, but further researches need more precise optimization to be carried out in a commercial scale. The life cycle assessment (LCA) should also be carried out by considering several environmental factors of NC biocomposites. BNC is particularly fascinating; however, this substitution is actually less feasible due to the limited scale up of BNC products to the commercial scale, which is linked to its existing huge cost of production of culture media and sluggish processing. Moreover, it is not only interesting to develop cleaner technologies for the production of NC from wood/plant biomass, which provides non-toxic and morphologically different products for specific pharmaceutical and biomedical industries. In the biomedical field, the innovations of biomaterials are also growing rapidly, so NC-based

biomaterials can be developed as a promising solution in the nearby future to overcome some challenges of other biomedical materials'. The extracts of numerous medicinal plants can induce many properties along with their biocompatible and non-toxic nature. So, to a great extent, these natural extracts in combination with NC will be explored in future. Its biocompatibility can be adjusted by surface derivatization for inducing hydrophobic, hydrophilic and partially water-soluble characteristics are especially encouraging.

In biomedical applications, the breakthrough is being made by NC-based hybrids containing biopolymers, bacteriocins, surfactants, enzybiotics and phytophenols with possible antimicrobial efficacy. There are frequent reports of noteworthy advances in biomedical NC-based composites, but this field is still in its infancy. There have been a variety of possibilities remains to explore in this subject about developing greener NC-based biocomposites for specific applications. This article demonstrated the current status, major challenges and commercial viability of NC- based biomedicine through the interpretation of preferred fields. NC biomaterials will certainly contribute to improving the quality of human life in the future from an economic and environmental friendly point of view, especially through the implementation of the next generation NC biocomposites.

Declarations

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Tables

Table 1: Specific characteristics of different types of Nanocellulose

Types of NC	Size (nm)	Length	Morphology	Crystallinity (%)	Aspect ratio	References
CNC	4 – 25	5-200nm	Rod/Needle	91.2	10-50	(Habibi et al. 2010; Lu and Hsieh 2012)
CNF	10 - 100	>1µm	Long chain	84.9	70-100	(Lavoine et al. 2012; Dufresne 2013)
BNC	20 -100	>1µm	Twisted Ribbon	70 -80	50-100	(Castro et al. 2011; Hu et al. 2013)

Table 2: Toxicology analysis of different nanocellulose materials

Cytotoxicity analysis	Effect	Reference
<i>Cytotoxicity analysis of NC from cotton using flow cytometry assay</i>	Lower NC concentrations of 0.02–100 µg/mL did not result in cell death. However, concentrations of >200 µg/mL resulted in cell death and alterations in mammalian fibroblast gene expression. The expression of various markers such as stress and apoptosis was impaired by higher levels of CNCs between 2000 and 5000 µg/mL.	(Pereira et al. 2013b)
<i>Cytotoxicity analysis of NC using different cell lines</i>	No toxic effect was observed at 0–50 µg/mL and with an incubation time of 48 h	(Dong et al. 2012)
Proliferation testing assay with 3T3 fibroblast cell line and Chinese hamster ovary cells using <i>in vitro</i> genotoxicity of CNF. Comet assay for DNA fragmentation	No cytotoxic effect was observed No DNA damage effect was observed	(Moreira et al. 2009)
<i>In vivo</i> and <i>in vitro</i> cytotoxicity analysis of NC in mouse model and human umbilical vein endothelial cells.	No cytotoxicity was observed in endothelial cells No physiological changes were noticed in mouse model.	(Jeong et al. 2010)
<i>In vitro</i> cytotoxicity test of NC on 3T3 fibroblast cell lines.	Tested material did not exercise any toxic effect on fibroblast cells lines. No noticeable changes on cell membrane, mitochondrial activity, or DNA proliferation.	(Alexandrescu et al. 2013)
<i>Genotoxicity analysis of NC</i>	No significant DNA damage	(Hannukainen et al. 2012)
CNCs digestion was analyzed using a three-phase simulator, and the subsequent digestas from	No cytotoxicity was observed	(Cao et al. 2020)

small intestinal phase were injected to an *in vitro* human cellular model of the SIE.

Table 3: Commercial status of NC-based products for biomedical field

SL. No.	Product/Trade name	Company(Country)	Applications	References
1	Biofill®	Human med AG; Fibrocel (Brazil)	Treat burns and ulcers	(Fontes et al. 2019)
2	XCell®	Xylose corporation(USA)	Treatment of venous leg ulcers	(Gama and Dourado 2018)
3	Gengiflex®	Organogenesis Inc. (USA)	Regeneration of periodontal tissues, guided bone tissue regeneration	(Pittella and Porto 2015)
4	Cellumed	Cellumed Co. Inc(South Korea)	Treat large surface wounds of animals	(Lee et al. 2015)
5	BASYC®	POLYMET Jena (Germany)	Artificial blood vessel, cuff for nerve suturing	(Frensemeier et al. 2010)
6	BioProcess®	Fibrocel Produtos (Brazil)	Skin transplant, treatment of third degree burns, ulcers, and decubitus	(Unal et al. 2020)
7	NexFill®	Fibrocel Produtos (Brazil)	Dry bandage for burns and wounds	(Ferreira et al. 2011)
8	DermaFill™	Cellulose Solutions (USA)	Translucent wound dressing	(Lina et al. 2011)
9	Suprasorb® Xb	Lohmann & Rauscher (Germany)	HydroBalance wound dressing for lightly to moderately exuding, noninfected wounds	(Heinze 2018)
10	CELMAT® Wound/Eye/Face	Bowil Biotech (Poland)	Hydrated wound dressing	(Ludwicka et al. 2016)
11	BioCelltrix	BC Genesis (USA)	Surgical mesh	(Cordeiro et al. 2004)
12	SyntheCel®	Synthes (USA)	Dura replacement devices (implants)	(Young et al. 2018)
13	Securian®	Xylos (USA)	Macro-porous surgical mesh	(Huertas and Matilla 2020)
14	Growdex®	UPM Biomedicals, (Finland)	Hydrogel for 3D cell culturing and other biomedical applications	(Joki et al. 2018)
15	<i>Nanocell</i>	<i>Thai Nano Cellulose Co Ltd, (Thailand)</i>	Wound dressing	(Muangman et al. 2011)
16	Nanoderm™	Axcelon Biopolymers Corporation (Canada)	Wound healing	(Ardila et al. 2016)

17	Bionext®	Bennett Health (USA)	Skin repair	(Joyeux et al. 2020)
18	Cuticell® – Epigraft –	BSN medical (Germany)	Wound dressing	(Dwiyana et al. 2019)
19	Epicite ^{hydro}	JeNaCell (Germany)	Wound dressing	(Vilela et al. 2019)
20	Membracel	Vuelo Pharma (Brazil)	Skin care	(Assis et al. 2017)

Figures

Figure 1

Properties of nanocellulose biomaterial for biomedical applications.

Figure 2

Applications of nanomaterials in different biomedical fields.