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Review

# Nanocrystalline cellulose: Preparation, physicochemical properties, and applications in drug delivery systems

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ABSTRACT

Cancer is the leading cause of death all over the world and chemotherapy is an important approach to fight cancer, however, there are many obstacles against successful cancer chemotherapy such as development of multi-drug resistance, poor solubility of chemotherapeutic agents and adverse side effects to healthy tissues. An important strategy to overcome these obstacles, is the use of nanotechnology. In recent years, natural polymers such as cellulose and its nanoform structure, nanocrystalline cellulose (NCC), have attracted the interest of researchers in the field of nanotechnology and specially drug delivery systems, due to biocompatibility and biodegradability of NCC. Cellulose is the most abundant natural biopolymer and changes to NCC by several chemical and mechanical methods. In this review, we mainly focus on the methods for production of NCC, physicochemical properties and medical applications of NCC (e.g. regenerative medicine, replacement of vascular grafts, tissue engineering, anti-bacterial/anti-viral applications, diagnosis and biosensing) with a special emphasize on drug delivery systems.



**Contents**



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## 1. Introduction

Cellulose is the most abundant biopolymer in the nature and has a variety of properties such as renewability, biodegradability, and non-toxicity. This polymer consists of six hydroxyl groups within the repeating structure of β-D-glucopyranose units, giving a high degree of functionality to the cellulose. Due to these advantageous characteristics, various structures and compounds have been made based on cellulose [1]. The first report about the production of nanocrystalline celluloses (NCC) from native and mercerized wood cellulose, and from viscose rayon was published in 1950 in which sulfuric acid catalyzed the degradation of cellulose. Production of NCC in acidic condition leads to the exhibition of the large surface area for presenting hydroxyl groups. In this condition, different chemical modifications can be done on its surface including esterification, oxidation, silylation, etherification, and polymer grafting [2].

In the field of medical sciences, NCC has attracted much attention; this biomedical nanocarrier is biocompatible and biodegradable composite and does not trigger immune system response. Surface area of NCC governed by hydroxyl groups can bind covalently or non-covalently to multiple functional groups and bioactive molecules. Furthermore, this rod-like nanocarrier has sizes ranging about 50–200 nm long. Due to these structural features, it cannot be removed by the kidney which increases the stability of nanoparticle in the body, but macrophages can easily remove it from the bloodstream in to the cells leading to strengthened performance of NCC [3]. In recent years, multiple researches have focused on the use of natural polymers in various fields of medicine including drug delivery, tissue engineering, and vascular grafting. Numerous NCC-based materials have been created for biomedical applications due to the biocompatibility and biodegradability of NCC [4]. In this review we focus on some important properties and applications of NCC, methods for the preparation of nanoscale cellulose, multiple surface modifications which increases the efficacy of drug delivery, targeting cancer cells based on NCC and chemical changes that occur at the surface of NCC for controlled release of drugs in acidic condition of cancer cells and many other biomedical features of NCC.

## 2. Methods for preparation of NCC

Nanocrystalline celluloses (NCC) comes from a variety of sources such as wood, flax, bacterial cellulose, tunicate, and microcrystalline cellulose (MCC). There are various methods for the preparation of these crystalline nanoparticles depending on the type of cellulose raw materials, their pre-treatment and their decomposition process. The most common method is hydrolysis by acid. The process of acid hydrolysis to produce NCC, has side effects such as destruction and corrosion of cellulose, and the most important of these effects are environmental degradation. Furthermore, hydrolysis by acid significantly reduces the thermal endurance of nanocrystals [5]. It has been observed that the NCC obtained by solid phosphotungstic acid hydrolysis have a higher thermal stability than those produced by sulfuric acid hydrolysis. Also, the presence of metal ions such as inorganic chlorides, $FeCl_3$-$6H_2O$, $CuCl_2$-$2H_2O$, $AlCl_3$, and $MnCl_2$-$4H_2O$ leads to increase the effectively acid hydrolysis process [6]. Therefore, finding a simple, environmentally friendly, and low cost method is a great challenge. Today, several mechanical processes for the preparation of nanofibers from cellulosic materials have been investigated and used, including high-pressure homogenization, pulping beating and cryocrushing. Recently, ultrasonic techniques have also been used to isolate and provide cellulosic nanofibers. Ultrasonication involves the use of sound energy to chemical and physical systems. The chemical effects of ultrasonication for the preparation of NCC are derived from acoustic cavitation. During the acoustic cavitation, the high pressure of the water stream, the potential energy of the bubbles is converted to kinetic energy and the bubbles collide at 100 m/s to the MCC surface and eventually a NCC occurs during this physical collision (physical stress). It has been shown that ultrasonication is a non-selective method that removes both crystalline cellulose and amorphous cellulose from the environment [5]. A recent study has reported that chemical methods of converting micro-fibrils to NCC are better than mechanical methods, because chemical methods are low cost-effective in terms of energy and also crystallization is better [7]. MCC is a nearly pure cellulose source, due to the high percentage of cellulose and high availability in laboratories; it is a good option for obtaining a NCC [5]. NCC isolation from cellulosic sources involves two important steps: (I) pre-treatment of cellulosic source, which is used to remove partial or total matrix materials such as lignin and hemicellulose. Finally, cellulosic fibers can be obtained. (II) The chemical treatment of cellulosic fibers is usually done by acid to remove amorphous regions. The pre-treatment step is performed in accordance with the cellulose source. For example, if the cellulose source is wood, then the wood parts are placed at temperatures of 200–270 °C and the pressure of 14–16 bars for 20 s to 20 min. Then, the pressure applied on the sample is immediately reduced (at atmospheric pressure) until a steam explosion occurs. During the explosion of steam, all non-cellulosic materials are decomposed in the sample and cellulosic fibers remain. In regard to the second step, which is the chemical treatment of cellulosic fibers obtained from the first step, Ranby is the pioneer in the production of crystalline cellulose suspensions from these fibers, which is carried out by hydrolysis with sulfuric acid. During hydrolysis by acid, the amorphous regions have not been resistant to acid and are hydrolyzed, but the crystalline regions remain resistant to acid and remain in the environment [8]. To obtain cellulose nanofibers from raw cotton, before sulfuric acid hydrolysis, to make the cotton completely dissolved, copper (II) sulfate 5% in distilled water followed by a sodium hydroxide (5 M) and ammonia (25 wt%) used, and finally, to obtain an aqueous suspension of cellulose nanofibres, sulfuric acid Hydrolysis used [9,10].

A comparison between the chemical and mechanical methods is shown in Table 1. NCC obtained from the combined hydrolysis of hydrochloric acid and sulfuric acid are spherical, while, the NCC obtained from ultrasonication are rod-like shaped. Spherical NCC have a better thermal resistance than rod-like NCC, because spherical NCC have fewer sulfate groups on their surface. In addition to acid hydrolysis, limited articles reported enzymatic hydrolysis for the preparation of NCC [2].

The enzyme hydrolysis is mainly used to produce microfibrillated cellulose (MFC), but it has been observed that after mechanical digestion, then acidic hydrolysis, and finally enzyme hydrolysis, a suspension is obtained consisting of MFC and NCC [8]. It has been shown that cellulosic nanomaterials obtained from composite methods have better properties in terms of their dimensions and temperature resistance. Furthermore, use of enzymatic hydrolysis in combination with

**Table 1**
Types of mechanical and chemical methods for preparation of NCC with their advantages and disadvantages. CNF: Cellulose nanofibrils, MFC: microfibrillated cellulose.

| Methods | | | Ref |
|---|---|---|---|
| Chemical methods | Acid hydrolysis | Advantages: Selective method (Just remove amorphous regions). Variability in NCC size due to randomized hydrolysis (length: 70–2000 nm and width: 3–50 nm). | [8] |
| | | Disadvantages: Having the potential for cellulose degradation, Reactor corrosion and environmental hazards | [5] |
| | Enzymatic hydrolysis | Advantages: The ability to produce NCC (rod-like) and MFC (length at micrometer scale and width: 20 nm and more flexible than NCC). | [8] |
| | | Disadvantages: Reduction of viscosity of particles. | [8] |
| Mechanical methods | Ball milling | Converting the micro to nano-scale and producing CNF. Simple operation. Use of cheap equipment. Extensive application capability for most cellulosic sources. Produce nanocellulose in large quantity at room temperature and pressure. | [11] |
| | High-pressure homogenizing | Production of cellulosic nano-scaled fibril networks and NCC (needle-like in length: 40–140 nm and width: 6–14 nm). | [12] |
| | Cryocrushing | Production of microfibrils with diameter ranging 0.1–1 μm. | [13] |
| | Ultrasonication | Non-Selective method (remove amorphous and crystalline regions). Reduce crystallization by increasing ultrasonication time. | [5] |

mechanical shearing and high-pressure homogenization results in the production of cellulosic nanomaterials with a scale of 5–6 nm. It has been shown that bacterial NCC obtained from enzyme hydrolysis (commercial cellulose enzyme) in combination with mechanical shearing have better mechanical and temperature properties than those obtained from hydrolysis by sulfuric acid [7]. The production of NCC from a crystalline cellulose suspension is as follows: 1- Hydrolysis of cellulosic fibers is carried out by 64% acid and with 10 ml/g and 45 °C for 1 h. (Acid concentration is not higher than 64% - the temperature is allowed from room temperature to 70 °C - Hydrolysis time depends on the temperature used for 30 min to overnight). 2 - Diluting the suspensions 10 times with cold water to stop the reaction, rinsing with deionized water at 4 °C for 10 min, and successively centrifuges at 4550 g. 3 - Extensive dialysis with deionized water to completely remove free acid molecules (dialysis is done as long as the pH does not change). 4 - A mechanical treatment, mainly sonication (with a power of 200 w by cold water), designed to produce sustained suspensions and homogeneous form of nanocrystals. Finally, the suspension is filtered by a 0.45 μm filter to remove any dense alloys from the environment. 5 - Concentration and drying of nanocrystalline suspensions to obtain solid NCC (Fig. 1) [8]. During drying process, the force generated by the removal of water from the suspension and also the high temperature of the environment may drive the molecular contact of NCC and cause agglomeration. Several methods have been studied for drying suspensions of crystals, which are described in Table 2 [8].

### 2.1. Properties of NCC

NCC, as one of the strongest and stiffest natural materials available, has significant properties, including high tensile strength, high hardness, large surface area [14], low density, high aspect ratio, changeable surface properties due to hydroxyl reactive groups, and other electrical, mechanical, thermal and optical properties [8].

#### 2.1.1. Thermal properties

Regarding the thermal properties of NCC, it should be noted that the degradation of the NCC or the reduction of their mechanical properties at high temperatures, is one of the major factors that limit the use of NCC [8], but in some cases NCC is a good candidate for use in processes like thermoplastics, that processing temperature is >200 °C [5], (NCC degradation, usually occurs at temperatures of 200–300 °C [8]).

Due to the unique properties of NCC, as described above, it has potential applications in some fields such as materials science, electronics, and medicine. For example, the use of NCC in the paper industry has produced paper with variations in paper surface permeability, strength

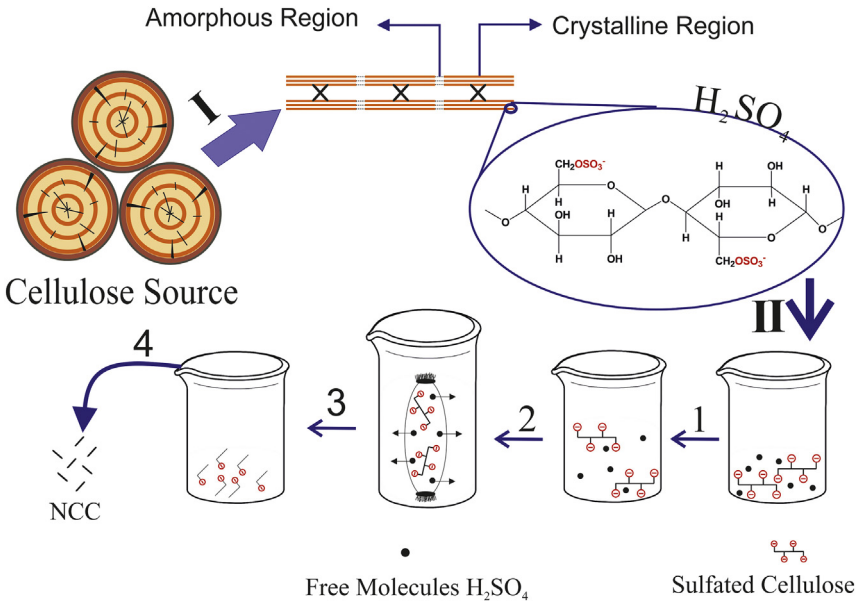


**Fig. 1.** Production of NCC from cellulosic source. Cellulosic source (such as, wood) was affected by the steam explosion process to remove non-cellulosic material, and remain only cellulosic fibers (**I**); By adding H2SO4, amorphous regions are removed and acid molecules reacted with hydroxyl groups of fibers surface, which causes the cellulose to be sulfated and the surface charge of fibers is negative (**II**); In this step, suspension includes cellulosic fibers with negative charge and free acid molecules. Diluted the suspension 10 times with cold water to stop the reaction, then rinse with deionized water, and successively centrifuge (**1**); Extensive dialysis with deionized water to completely remove free acid molecules (**2**); Mechanical treatment, mainly sonication, designed to produce sustained suspension and homogeneous form of nanocrystals (**3**); drying of nanocrystalline suspension to obtain slide NCC (**4**).

**Table 2**
Types of methods for drying cellulose suspensions and the final size of obtained particles.

| Methods for drying | Particle dimension |
|---|---|
| Oven | Micron or even to Millimeter |
| Freeze drying | Nano |
| Supercritical drying | Nano |
| Spray drying[a] | Nano and Micron |

[a] It is less costly than other drying methods.

and flexibility. Furthermore, in some cases NCC creates special papers with optical properties [15].

*2.1.2. Optical properties*

For the first time in 1959, Murchessault et al., observed liquid crystalline of NCC with birefringence of suspensions, which led to the observation of the NCC interesting optical properties, and attracted the focus of most studies on this phenomenon. With regard to the optical properties of NCC, Revol, Godbout, and Gray created solid iridescent cellulosic films with unique NCC properties and by controlling evaporation of water on a flat surface, for the production of security papers such as banknotes, ID cards and passports [5].

*2.1.3. Mechanical properties*

To calculate the NCC tensile properties, theoretical calculations and indirect experimental measurements using atomic force microscopy (AFM), X-ray diffraction analysis, inelastic X-ray scattering, and Raman scattering were used. Theoretically, the tensile strength of the NCC is much higher than steel wires [7]. NCC has been studied extensively in various fields, including polymer electrolytes, packaging, antireflective materials, and solid iridescent films, due to their excellent mechanical properties [16]. Finally, not only NCC inherent properties of natural cellulose, but also have unique properties such as high crystallinity index (>70%), large surface area (~150 $m^2/g$), big aspect ratio (~70), and high tensile strength (7500 MPa). In recent years, these properties have caused NCC to be considered in some fields, including regenerative medicine, printing applications, optical application, and composite materials [17].

*2.2. Surface modification of NCC for drug delivery*

The particle has potential properties as a drug carrier including a very large surface area and a negative surface charge (due to the numerous hydroxyl groups on the NCC surface). These features make it possible to attach a large amount of drugs to the NCC surface with optimal control of dose [18]. The characteristics of the drug delivery systems such as particle size, surface charge, modification and biocompatibility have an important effect on drug release and delivery process of the drugs [19]. NCC as a drug carrier in biomedical applications, have two very important properties: I- NCC has a taller length than other nanoparticles, and thus long lasting for the removal by the renal system. On the other hand, these particles are small enough to delay their clearance rate from the bloodstream. II- The hydrophilic nature of NCC surface, prevents the absorption of opsonin protein (a vital step prior to phagocytosis), therefore, the half-life of NCC in the circulatory system is higher than the hydrophobic nanoparticles. Surface chemistry of NCC is controlled by hydroxyl groups, which can be modified to other functional groups [3]. Due to the exceptional physical properties of NCC to enter cells, they have been investigated for drug delivery systems. Various chemical modifications of NCC can be performed on hydroxyl groups of crystalline surface. These modifications include sulfonation, oxidation, cationization, silylation, esterification, carboxylation, and carbidation [7,14,20]. From the three groups of hydroxyl on the NCC surface, the hydroxyl group on the sixth position, acts as the first alcohol [20]. These chemical modifications as linker bind the nonionized or hydrophobic drugs to the surface of NCC, which in normal conditions, do not bind to unmodified NCC [14]. Advances have been made in the use of modified NCC for new biological applications such as immobilization of enzymes, green catalysts, antimicrobials, and controlled drug delivery during treatment and diagnosis in medical applications [7,14]. Treatment process includes delivery of therapeutics by modified NCC to the tumor cells and diagnosis process includes the use of contrast agents for magnetic resonance imaging (MRI) [21]. Because of the high surface-to-volume ratio and the negative surface charge, many drugs can be loaded onto the surface of NCC with the potential for optimal control of concentration [7]. The problem with the use of cellulose in nanocomposite applications is the poor compatibility of the hydrophilic NCC with the hydrophobic matrix. The performance of these nanocomposites is heavily dependent on the interaction between hydrophilic NCC and the hydrophobic matrix phase [22]. It is also a big challenge to maintain the morphology of NCC crystals in the modification process [7]. NCC modifications improve the binding of hydrophobic drugs on the NCC, and dispersion of these particles in a wide range of organic solvents. There are two general methods for these modifications: I. Covalent chemical modification of NCC surface such as oxidation, esterification, acetylation, silylation, cationization and II. Use of surfactant-coated NCC suspensions [8]. The common approach used for chemical modification involves the attaching of single functional organic molecule (such as ASA, EPTMAC, and TEMPO) and surfactants such as CTAB and CSos to the hydroxyl groups of NCC to increase the hydrophobicity of particles [22]. Due to the increased efficiency of modified NCC in important industries such as personal care and biomedicine, much research has focused on NCC modification. Yuan et al. (2006) for acetylation used straightforward freeze drying and heating aqueous emulsion of alkenyl succinic anhydride (ASA) and NCC suspensions to obtain acetylated NCC, and Braun and Dorgan (2009) synthesized NCC with acetic acid, HCl and other organic acids, and then it was esterificated by Fischer method. Alkyldimethylchlorosilanes is a solution that silylation process was also performed and finally Gray et al. (2008) described the cationization process by activating the hydroxyl groups of the NCC surface by alkali and linking these groups with epoxypropyltrimethyl ammonium chloride (EPTMAC). Oxidation is the conversion of hydroxyl groups to carboxylic acid by the 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) reagent. During oxidation, TEMPO is a nitroxyl free radical for the initial oxidation of hydroxymethyl in the presence of NaBr and NaOCl [20].

Furthermore, some surfactant-coated NCCs with CSos and CTAB, are mentioned:

A new modification method to produce chemically grafted chitosan oligosaccharide (CSos) on NCC was developed. In the first step, the primary hydroxyl groups were oxidized selectively by TEMPO-mediated to carboxylic acid groups. The amino group of the CSos then reacts with the carboxylic acid group on the surface of the NCC by carbodiimide. NCC-CSos were loaded with drugs such as Procaine hydrochloride (PrHy) (hydrophobic drug). Studies in drug release in pH = 8 revealed that the drug was released from the nanoparticle for 1 h. These modified NCC have potential applications such as fast response drug carriers and local drug delivery to the oral cavity, such as treatment of periodontal cavities [23]. Chitosan is used with NCC in the drug delivery systems because of its positive charge on amino groups of sugar that firmly bind to the negative charge of drugs such as antisense oligonucleotides [14].

A study was conducted to encapsulate hydrophobic and hydrophilic drugs on unmodified particles and surfactant-modified particles. In a study, two groups of hydrophilic (Doxorubicin hydrochloride (DOX) and tetracycline (TET)) and hydrophobic drugs (paclitaxel (PTX) and docetaxel (DTX)) were studied. Since DOX was a cationic drug and TET was a zwitterionic drug, they were easily attached to the negative charge of NCC surface (unmodified NCC). In in vitro conditions, these drugs were rapidly released from the NCC. This rapid release of the drug from the NCC is used to treat cancer by the removal of the local tumor. In addition, there is a solution to deliver hydrophobic drugs such as PTX and DTX by NCC. In this way, the negative surface charge of NCC was modified by

cationic surfactant called Cetyl trimethylammonium bromide (CTAB), and the hydrophobic property was given to the NCC. Then, hydrophobic drugs were loaded onto the CTAB's domains on the NCC. The results showed that hydrophobic drugs on modified NCC are released more slowly than hydrophilic drugs on unmodified NCC. This has been proven by studies on KU-7 cancer cells [18,23]. These modifications increased the surface hydrophobicity of NCC and also caused the binding of hydrophobic drugs to modified NCC. [20].

Among the two general methods used for NCC modification, it seems that the chemical modification method is more beneficial, since in this method the mechanical properties of nanoparticles are preserved, although, this method is expensive. However, surfactant-coating is still used to modify the NCC [23].

Finally, nanomedicine is known for targeted delivery of drugs to treat a wide variety of diseases. This perspective focuses only on cancer-based nanomedicine treatments, since two-thirds of studies have focused on this issue. The fact that modifying nanomedicine improves the therapeutic index of anticancer drugs, is well-known and clinically proven [19].

### 2.3. Polymeric pH-sensitive NCC

Polymeric nanoparticles have emerged and developed as a progressive and pioneer technology for controlled and targeted drug delivery systems in past decades. These nanoparticles dissolve, swell and collapse in response to internal stimulants like pH, redox potential and lysosomal enzymes to increase the efficiency of drug release at the target site of cancer cells. Acid conditions of cancerous cells (pH 6.5–7.2) and dominant pH on endosomes (pH 5.0–6.5) and lysosomes (pH 4.5–5.0) are of the conditions where nanoparticles can function in a specific way [24]. pH sensitive nanoparticles have been designed and developed for specific drug delivery to the tumor sites and endo/lysosomal compartments [25]. NCC has received a great deal of attention in the last decades. In addition to biocompatibility and biodegradability, the presence of numerous hydroxyl groups in the surface of NCC make this nanoparticle as a promising drug delivery vector. These hydroxyl groups make the surface of the nanoparticles highly reactive and chemical agents can be added to its surface. For example, one of these reactions is the conversion of the hydroxyl groups of NCC to the carboxylate nanocrystalline cellulose (CNCC) [26]. Due to the existence of carboxylic acid groups in CNCC, it shows different features in various pH conditions. pH sensitivity of CNCC enhanced significantly by increasing the number of carboxyl groups added to the surface of NCC. Based on the pH of the medium, the $–COO^-$ and –COOH as functional groups in the surface of CNCC, can be interchanged. This carboxyl group has a $pK_a$ of about 3.2. When the pH of the medium is lesser than $pK_a$, most carboxyl groups are in –COOH state, and strong hydrogen bond formed between –OH of NCC and –COOH, which can prevent the water from spreading into its structure. In contrast, when the pH of the medium is higher than $pK_a$, most carboxyl groups are in $–COO^-$ state, and this situation leads to a significant reduction in the hydrogen bound. Due to the increased negative charge, the electrostatic repulsion in the surface of CNCC became dominant, which leads to the penetration of water into the nanoparticles [15]. The in vitro drug release studies was carried out at different pH values in order to study the release of drugs in conditions similar to cancer cells. Results showed that in highly acidic (pH 4) conditions, drug release from NCC is weak, but when the pH was enhanced, the drug release from nanoparticles explosively increased [27]. These sophisticated nanoparticles which respond to stimuli such as pH, designed to enhance the augmentation of multiple drugs such as chemotherapeutic agents [28]. The production and enhancement of pH sensitive nanocrystalline cellulose is very important in treatment of cancer cells. Because the pH in the environment of cancer cells is different from normal cells, it is important to design a drug delivery system that can remain soluble in aqueous environment of cancer cells. Surface modification of NCC by 4-vinylpyridine (P4VP-g-NCC) leads to production of pH responsive polymer. This smart modified NCC changes its character from hydrophobic to hydrophilic when the pH of environment changes. At pH values under 5, the 4-vinylpyridine group is protonated and placed in hydrophilic state and dispersed in aqueous environment of cancer cells while, at pH values above 5 the 4-vinylpyridine group is deprotonated and as a result, its dissolution in the environment around the cancer cells is reduced [29]. The smart design of a nanoparticle, such as a P4VP-g-NCC based on the metabolic characteristics of cancer cells, increases the efficiency and effectiveness of cancer treatment and also suggested that NCC based nanoparticles with the ability of respond to pH should be further studied, since the NCC is biodegradable compound and has no toxic effect in the body. Furthermore, due to the presence of abundant hydroxyl groups on the surface, varied reactive agents can be placed on NCC (Fig. 2).

### 2.4. Biomedical applications of NCC

Because no toxicity has been introduced for NCC, several research groups have introduced NCC for use in biomedical applications. Dong et al., proposed modified NCC in targeted delivery of drugs, especially chemotherapeutic agents [30]. For example, targeted and controlled delivery of folic acid for cancerous tumors of the mammalian brain by NCC was successful. In addition to the drug delivery process, NCC have many biomedical applications such as the process of diagnosis and biosensing (e.g. the use of NCC to detect the human neutrophil elastase enzyme at the site of injury) [31], replacement of vascular grafts (in cardiovascular diseases) [32], enzyme/protein immobilization [33], viral inhibitors (alphaviruses, herpes simplex viruses, and perhaps AIDS virus) [34], tissue engineering (TE) [35] and bacterial inhibition (inhibition of *Mycobacterium smegmatis* and *Staphylococcus aureus*) [36], which is described in detail. It should be said that the fluorescent labeling of NCC provides potential applications in biomedical issues including bioprobes, fluorescence bio assay, and bioimaging applications [7] (Table 3).

#### 2.4.1. Diagnosis and biosensing

Human neutrophil elastase enzyme (HNE), a kind of serine protease, is released at high concentrations by neutrophils at the site of chronic injury, causing the breakdown of growth factors and connective tissue proteins, which ultimately results in poor wound healing and worsening of injury. The pathology of non-healing injuries leads to the production of protease-lowering dressings, which 1- binds proteases (e.g. elastase) to

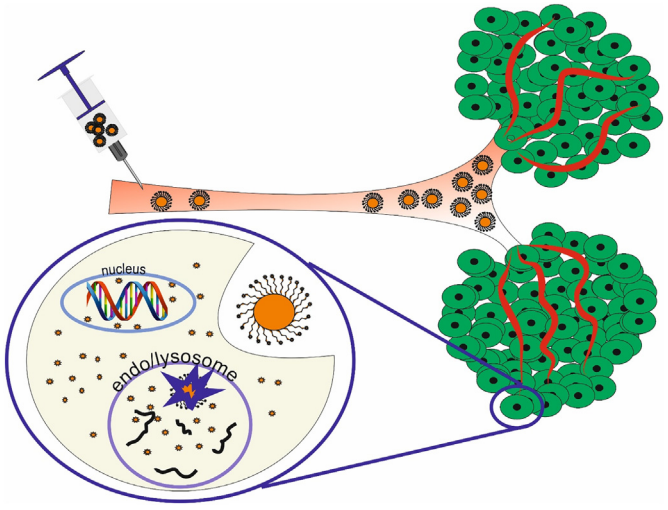


**Fig. 2.** Schematic representation shows the drug release from NCC in the high acidic condition of cancer cells, the intracellular environment including the high acidic situation in the cytoplasm and endo/lysosomal compartment increase drug release from NCC inside tumor cells.

**Table 3**
The most prominent properties of the NCC with their functions and their final efficiency.

| Properties | Functions | Results | Toxicity | Ref |
|---|---|---|---|---|
| Diagnosis and biosensing | CNC peptides conjugates, detect and measure proteases such as elastase in chronic injuries. | To treatment of chronic injuries, produce protease-lowering dressing with CNC. | Negative | [37] |
| Replacement vascular grafts | Covalent coupling between CNC and fibrin matrix to produce synthetic nanotubes. | This nanocomposites, replacing vascular graft surgery in cardiovascular diseases. | Negative | [32,33] |
| Anti-viral | Unmodified and modified CNC have anti-viral property. | CNC inhibited alphaviruses and maybe HIV and herpes simplex viruses. | Negative | [34] |
| Tissue engineering | CNC is suitable to produce tissue engineering scaffolds. | Collagen, elastin and etc. scaffolds for skeletal muscle or tendons. | Negative | [33,35] |
| Anti-bacterial | Synthesis a new anti-bacterial agent with CNC. | CNC/porphyrine, inhibited *Mycobacterium smegmatis* and *Staphylococcus aureus*. | Negative | [33,36] |
| Enzyme/protein immobilization | Immobilized enzyme with CNC, increased the catalytic activity of enzyme and improved affinities of enzyme-substrate. | CNC/peroxidase removed chlorinated phenolic compounds from aqueous solutions better than free peroxidase. | Negative | [33,40,41] |

the dressing surface, thereby removing elastase from the wound surface or 2- inhibiting proteases such as elastase. Therefore, the use of NCC-peptides conjugates was introduced as a biosensor for detecting and measuring of HNE. The diagnosis and timely measurement of HNE in both in situ wound dressing sensor and prognostic biomarker manner is recommended for the healing of wounds [37].

*2.4.2. Replacement of vascular grafts*

NCC as blood vessel replacement study is attractive to most researchers, because the results of this process have been proven in various animals before clinical research. One of the most commonly used treatments for cardiovascular diseases is coronary artery bypass surgery, which has recently developed nanocomposites using NCC and fibrin, replacing vascular graft. Covalent coupling between NCC and fibrin matrix, produces synthetic nanotubes with proper strength and elasticity, which are used as a replacement for vascular graft surgery. These nanotubes have not been tested under in vivo conditions [32]. Both native cellulose and fibrin have been investigated individually in the process of replacement of vascular graft surgery, but both had deficiencies. For example, cellulose possessed a limited elongation and fibrin showed insufficient strength, however, since NCC is equivalent to collagen and fibrin is equivalent to elastin, the NCC/fibrin nanocomposites, induces the collagen-elastin condition [38].

*2.4.3. Anti-viral property*

The use of NCC as substrates for fluorescent imaging and targeted drug delivery such as tetracycline, doxorubicin, docetaxel, and paclitaxel, has already been presented, however, there are very limited reports about the use of NCC as viral inhibitors. Unmodified particles have been found to be viral inhibitors of alphaviruses. Interestingly, modified NCC containing the tyrosine sulfate groups, exhibits more inhibitory effects. Therefore, the sulfate groups on the surface of the NCC, were removed and the phenyl sulfonate groups were added to the NCC surface with 4-sulfophenyl isothiocyanante (4-SPITC) in an aqueous medium or through a three-step protocol in an organic medium. Observations have shown that the incorporation of specific characteristics of target viruses with NCC surface, regulates and controls anti-viral activity of NCC. It is believed that NCC have the potential to inhibit other viruses such as HIV and herpes simplex viruses [34].

*2.4.4. Tissue engineering*

However, NCC studies over the past two decades have been rising, the interest of researchers in the use of NCC in the field of TE, has recently increased. Tissue engineering scaffolds have features that affect their function. Therefore, attention to the composition and spatial structure of the components of these scaffolds (collagen, elastin, protoglycane, and adhesion molecules) in various tissues of the body is important and necessary. According to researches, NCC has been introduced as a reasonable candidate for TE, because NCC has the smallest features for directing contact between mammalian cells (even several times smaller than myoblast cells). According to the above, NCC is used in tissue engineering, such as skeletal muscle or tendons [35].

*2.4.5. Anti-bacterial property*

Sustainability and survival of pathogenic bacteria, especially antibiotic-resistant bacteria, lead to simultaneous transmission to new hosts and proliferation of pathogens, puts the human health at risk. Therefore, it is essential to synthesize a new antibacterial agent against a wide range of bacteria. In a recent study, the use of modified NCC with cationic porphyrin, (cationization by Cu (I)), the photodynamics of some bacteria such as *Mycobacterium smegmatis* and *Staphylococcus aureus*, to be inactivated [36]. In new studies, $TiO_2$ is used as an active ingredient for drug delivery such as antibiotics for antibacterial application, which has been observed as an interface between antibiotics and cellulose nano-fibers, lead to controlled and long-term release and drug delivery [9]. Also, antibiotics of triclosan, Tetracycline and Phosphomycin against *S. aureus* and *E.coli* in the presence of the $TiO_2$ (CNC/$TiO_2$/triclosan), caused long-term and controlled release during 3.5 h and with 83% release in the first 10 min [10,39]. It has been proved that cellulose nano-fibers modified only with $TiO_2$ have very high photocatalytic properties [10].

*2.4.6. Enzyme/protein immobilization*

Nanobiocatalysts are the enzyme and nanocarrier compounds, that attract more researchers due to their high catalytic performance, increased susceptibility, improved enzyme-substrate affinities and reusability [40]. Recently, due to the characteristics of the NCC such as surface-to-volume ratio, high aspect ratio, hardness and high strength, negative surface charge, non-porous structure and abundant hydroxyl groups, it is considered as a novel and interesting matrice for immobilization of enzymes/proteins [41]. Studies on immobilization of enzymes using NCC were started in 2008. The enzyme/NCC can significantly increase the catalytic activity, stability and enantioselectivity of the enzymes. In addition to that, enzyme-based processes replaced traditional methods in the lab and industry due to their high efficiency and their environmentally friendly properties [40]. For the first time, NCC was used as an enzyme support for peroxidase immobilization. During this experiment, peroxidase was used to remove chlorinated phenolic compounds from aqueous solutions. NCC was activated by cyanogen bromide before coupling to the peroxidase. The results showed that the activity of peroxidase coupled with NCC is higher than free peroxidase and the catalytic efficiency of the NCC/peroxidase is higher in the removal of chlorinated phenolic compounds [33,40].

*2.5. Cancer target therapy based on NCC*

Cancer therapy has become one of the main challenges in biomedical fields worldwide. Conventional cancer therapies, including chemotherapy, radiotherapy, and surgery, have serious side effects which harm normal tissues in addition to the tumor cells [42]. In recent years,

utilization of nanostructured systems for biomedical applications has been generally paid attention. Besides, with the advent of nanotechnology combined with recent advances in material sciences and the use of nanoparticles in medicine, more increasing interest for NCC as a desirable biomaterial with exceptional physicochemical and biological properties has emerged [43]. The manipulation of NCC may provide great advantages due to its large availability, complete renewability, physicochemical properties such as high surface area, unique morphology, mechanical strength, low density, and relatively low cost of production. The lack of obvious toxicity and untargeted cellular uptake of NCC, could candidate these nanoparticles as suitable carriers in drug delivery applications. Some efforts have been made to use NCC as a suitable pharmaceutical excipient or carrier. In the recent years, several procedures for surface modification based on the chemical introduction of different functional groups have been described. Some of these strategies are specifically devoted to the conjugation of drugs or targeting agents [44]. NCC has an abundance of hydroxyl groups which are easily converted to functional groups to conjugate drugs, imaging labels, and targeting ligands, such as folic acid. It is widely known that many cancer cells overexpress folate receptors in their plasma membrane which have a high affinity for folic acid. Dong et al. reported folic acid-conjugated NCC for the targeted delivery of chemo-therapeutic agents to folate receptor-positive cancer cells. Initially, NCC was labeled with fluorescent FITC for cell uptake evaluation. After labeling, in the presence of *N*-hydroxysulfosuccinimide (SulfoNHS) and carbodiimide (EDC), fluorescent NCC reacted with folic acid (FA). The folate receptor is overexpressed by several cancer cells and is likely to bind to FA. As expected, in the absence of FA, a lack of binding/uptake was observed. However, considerable binding/uptake of FITC-NCC-FA was reported. The later was significantly inhibited in the presence of free FA since it is the biological ligand for the folate receptor. It was clear that the folate receptor mediates FITC-NCC-FA uptake by cancer cells. So, cellular uptake of the conjugate was increased significantly compared to that of non-targeted NCC. By exploiting this fact, they suggested they can produce particles which target specific areas [30,45,46].

Based on the fact that there are abundant surface hydroxyl groups on NCC, Jackson et al. investigated the potential of NCC as a drug delivery excipient. Their results demonstrated that NCC was capable of binding to the significant quantities of ionizable water-soluble antibiotics such as tetracycline and doxorubicin. It was shown that the surface of NCC could be modified by binding to the cationic surfactant, cetyltrimethyl ammonium bromide (CTAB). CTAB-coated NCC was shown to bind to the significant quantities of the nonionized hydrophobic anticancer agents such as docetaxel, paclitaxel, and etoposide. The NCC/CTAB nanocomplexes bound to bladder cancer cells and demonstrated efficient delivery of a hydrophobic fluorescent probe fluorescein to the cytoplasm of these cells. Overall, these studies have established the potential of NCC as a drug delivery excipient for use alone or in conjunction with other formulations [46–48]. Aside from abundant hydroxyl groups, the surface of NCC may contain other types of functional groups that are directly related to its preparation and processing conditions. The common functional groups are sulfate groups and carboxyl groups. With additional post-hydrolysis reactions, aldehyde groups, amino groups or thiol groups may also be introduced to the NCC surface. Depending on the specific functional groups on the surface, NCC nanoparticles exhibit different charge properties. Additionally, the sizes of the cellulose nanocrystals in suspension can be controlled by a mixture of acid components, such as sulfuric acid and hydrochloric acid. This alteration is normally done to increase the adsorption capacity of NCC towards hydrophobic drugs into tumor cells [49]. In 2015, Hu et al. prepared poly (2-dimethyl amino ethyl methacrylate) (PDMAEMA) brushes on NCC using surface-initiated atom transfer radical polymerization from the initiation sites with disulfide bonds. It was reported that PDMAEMA can effectively condense DNA into nanoparticles to facilitate cellular internalization for gene delivery. Thus, it could be possible to design new NCC-based gene carriers if NCC is wrapped with dense PDMAEMA brushes. Disulfide bonds can be reduced by reducing reagents such as glutathione (GSH), in tumor tissues. The bioreducible PDMAEMA functionalized NCC showed higher transfection efficiencies, lower cytotoxicity as well as good activity in suppressing the growth of cancer cells [50–52]. In another study, a prodrug was prepared by the covalent attachment of tosufloxacin tosylate (TFLX) onto the surface of maleate-modified cellulose nanocrystals (MA-NCCs) with L-leucine as a spacer molecule. It was investigated that TFLX-AMA-NCC simulates colonic fluid (SCF), gastric fluid (SGF) and intestinal fluid (SIF). The relation between the accumulative drug release and the fluorescence response was evaluated. The results showed that the drug was efficiently entrapped by the MA-NCC carrier and presented excellent behavior for colon specificity. This engineered system might be considered as an applicable material for a colon-specific drug delivery system [53]. Moreover, NCC conjugated with rhodamine B isothiocyanate (RBITC) and with FITC (NCC-FITC) acting on HEK-293 (human embryonic kidney 293) cells was evaluated. NCC–RBITC, as a positively charged material, was taken up by these cells, causing no damage to cell integrity. No cytotoxic effect of these conjugates was observed. In contrast, the negatively charged material NCC–FITC underwent significant cell internalization at pH ~ 7. However, the surface charge of NCC exhibited an important role in either cellular uptake or cytotoxicity. The simple modification of the NCC surface might be in accordance with cell penetration without leading to cellular damage [54]. Generally, cancer target therapy based on NCC is a new type of therapy, which promises minimal invasive localized treatment of cancerous tissues, with little to no side effects.

### 2.6. Cellulose nanocrystals as drug carriers in medicine

The lack of obvious toxicity of NCC could candidate these nanoparticles as suitable carriers in drug delivery applications. Some efforts have been made to use NCC as a suitable pharmaceutical excipient or carrier.

As mention above, Jackson and coworkers reported that, CTAB-surface modified NCC could carry a significant amount of the hydrophobic anticancer drugs, docetaxel, paclitaxel, and etoposide and release these drugs in a controlled manner over 2 days. Due to its chemical property, CTAB might interact with the phospholipid bilayers of the cells, leading to destabilization of the cell membrane. This destabilization might result in cell death. Depending on its concentration, CTAB might create holes in the phospholipid bilayer of the cells. The authors observed uptake of NCC–CTAB complexes by KU-7 bladder cancer cells [55]. In order to improve the drug loading capability, the surface of NCC was modified with a cationic surfactant cetyltrimethyl ammonium bromide (CTMAB), due to the existence of anionic sulfate groups on the surface of NCC. Furthermore, it is possible to create a hydrophobic domain on the surface of NCC by coating the negatively charged NCC with CTMAB, and as a result hydrophobic water-insoluble anticancer drugs luteolin (LUT) and luteoloside (LUS) partitioned strongly into the CTMAB domains on NCC using free drug solutions at low concentrations. LUT and LUS release were found sustained over one day. Overall, this method established a novel drug delivery system which is potential to modulate the loading and release of drugs [56].

Recently, NCC with high mechanical strength and hydrophilic hydroxyl groups, has been used in accordance with the hydrophobicity and drug release of electrospun polylactic acid (PLA) nanofibers. PLA nanofibers exhibit fewer polar groups (high hydrophobicity) and high crystallinity, leading to low drug loading capacity of hydrophilic drugs. Polyethylene glycol (PEG) is a good compatibilizer for PLA and has been used to develop functional composite nanofibers for effective drug delivery. Moreover, the hydroxyl groups of PEG can interact with NCC, that has a good affinity to drugs [57,58]. As expected, the incorporation of NCC/PEG could improve the attachment of the cells on PLA/NCC/PEG, and opens the way for a new strategy for drug carriers into tumor cells. Electrospun PLA/NCC/PEG nanofibers were successfully prepared via electrospinning technique. Furthermore, the composite nanofibers

showed good biocompatibility with MG-63 cells by incorporating NCC/PEG and drugs. The composite nanofibers with improved physical and hydrophilic properties, especially longterm drug release, displayed good biocompatibility and exhibited great potential for application in fields such as long-term sustained drug delivery systems and other biomedical materials [59]. Besides, a folate group was bound to carboxymethyl cellulose nanoparticles conjugated with quantum dots in order to obtain specific recognition of cancerous cells. The anticancer agent 5FU was encapsulated in this nanoparticles for targeting cytotoxicity to MCF7 cells, compared to non-cancerous L929 cells. As expected, the epifluorescent images revealed certain specificity in the internalization of folate-conjugated nanoparticles, since the conjugated nanoparticles were internalized more in MCF7 cells due to the presence of FR in cancer cells, compared to non-cancerous L929 cells [53]. Akhlaghi and coworkers used oxidized NCC by modification with 2,2,6,6-tetramethyl-1-(pyperidinyloxy) (TEMPO), and chitosan oligosaccharide-grafted NCC as potential drug delivery carriers for two model drug compounds, imipramine hydrochloride (IMI) and procaine hydrochloride (PrHy). The results showed that IMI displayed higher binding to NCC derivatives than PrHy, although, drug binding and loading efficiencies for procaine hydrochloride on chitosan oligosaccharide-oxidized NCC were found to be 21.5% and 14% *w*/w, respectively. At pH 8, the release of procaine demonstrated a large initial burst phase. The initial burst was followed by a significantly slower release phase of the drug over the next hour. The authors suggested that the chitosan modified NCC might be suitable carrier for drugs in the condition that high drug release in a short time frame is desirable [23,60]. Wang et al. synthesized novel poly phosphor ester grafted cellulose nanocrystals. The well-defined propargyl-terminated PEEP was conjugated onto the surface of azide-modified NCC. The NCC-PEEP possessed a negatively charged surface which can be used to bind antitumor drug doxorubicin (DOX) and deliver the drug into the HeLa cells. The results of the MTT assay indicated that this NCC-based material showed good biocompatibility to both HeLa and L929 cells, while, the DOX-loaded NCC-g-PEEP nanocrystals exhibited a desirable anticancer activity only against HeLa cells. Furthermore, the intracellular drug release observed by a live cell imaging system demonstrated that these DOX-loaded nanocrystals could be internalized into HeLa cells through endocytosis and DOX could be released because of the disruption of the electrostatic interaction in the acidic environment inside the tumor cells [42]. It is known that curcumin, a polyphenol derivative, is the main bioactive constituent extracted from the rhizomes of *Curcuma longa*. It possesses many biological and pharmacological properties, including anti-inflammatory, antioxidant, anti-Alzheimer, anticoagulant and anticancer activities. Nevertheless, potential properties of free curcumin are hampered by poor solubility due to its hydrophobicity and low bioavailability combined with low stability in the solution that results in rapid clearance from blood [61]. Ntoutoume et al. reported that Cur–cationic cyclodextrins/NCC complexes are three to four times more effective than curcumin alone or Cur–CD complexes. CD/NCC nanocarrier alone, only slightly reduced cell proliferation at 48 h showing that antiproliferative effects observed on cells was actually due to the nanoconjugate-complexed curcumin. Thus, for an effective curcumin-based anti-cancer therapy, it is extremely important not only to increase its solubility, but also to encapsulate it in nanocarrier system which can transport curcumin into vascularized tumors via enhanced permeation and retention (EPR) effect. Their results showed that curcumin–CD/NCC complex was particularly effective against colorectal and prostatic cancer cell proliferation. CD/NCC had sizes about 200 nm which is suitable for drug loading and release [62]. Moreover, surface modification appears to be a suitable strategy for the design of versatile nanomaterials for drug delivery systems.

### 2.7. Oral administration of cellulose nanocrystals

Nanocrystals have emerged as a feasible tool to increase oral bioavailability of poorly water-soluble drugs [63]. Cellulose nanocrystals are essentially biodegradable and show good biocompatibility, suggesting that the oral administration of such particles is potentially safe. Surface functionalization of NCC is simple and a diversity of compounds can be anchored to the particles surface for altering their hydrophilicity and interface adsorption properties [64].

In the mouth, NCC is mixed with saliva. While positively charged nanoparticles may bind to salivary proteins and form “protein corona”, the negatively charged NCC is disgusted by the proteins [65]. In following to oral processing, NCC moves down by the peristalsis through the esophagus into the stomach, where the gastric acid and enzymes cannot trigger their breaking. NCC particles are unlikely to reach the gastric epithelium since, in the stomach, mucus secretion rate is so high that prevents non-mucoadhesive objects deposition and mucosal infiltration. Whereas, in the small intestine, mucus secretion is relatively slow which may allow NCC to penetrate the mucus layer and reach the epithelium. Due to the lack of cellulase enzyme in human small intestine, it is concluded that NCC based materials are not degraded significantly following the oral administration. Moreover, it is important to note that the pH variance in different gastrointestinal compartments can affect the aggregation status and surface chemistry of nanoparticles [64]. The gastrointestinal epithelium is covered by a mucus layer, which contains various proteins, including mucin and antiseptic proteins, such as lysozymes. Mucus in the oral cavity has a pH of about 6.6, whereas the pH of stomach mucus ranges from 1 to 2 at the luminal surface to about 7 at the epithelial surface [66]. In 2014 O'Connor et al. demonstrated that there is no indication of oral toxicity resulting from acute, sub-acute or chronic exposure to NCC. Exposure to NCC produced via the sulfate process in rats showed no adverse effects including morphological and behavioral changes at the highest dose examine [67].

Generally, current studies of the oral toxicity of NCC have shown a lack of adverse health effects. Additional studies are needed to support the general conclusion that NCC are nontoxic on ingestion.

### 2.8. Toxicity of NCC

In recent years, nanoparticles like NCC have become very much considered in the field of bio-applications due to their low toxicity [35]. The use of nanoparticles obtained from natural biopolymers (e.g. cellulose), is a substitute for inorganic nanoparticles due to their biodegradability and low toxicity [34]. From the point of view of toxicology, cellulose-based ingredients have shown excellent biocompatibility, particularly NCC that exhibit very little cytotoxicity in a wide range of human and animal cells. Various studies have shown that NCC at high concentrations exhibits cytotoxic responses that are significantly lower than the toxic concentrations of carbon nanotubes and crocidolite asbestos. This has led to a greater incentive for the use of NCC in various applications [35]. One of the major applications of nanoparticles is the drug carrier substances. To evaluate whether NCC can act as a drug carrier, the toxicity of NCC measured with human brain endothelial cells and finally showed no toxicity. Furthermore, studies on cellular uptake of NCC by the fluorescein-5′-isothiocyanate (FITC), reported the minimum non-targeted NCC uptake. Eventually, due to a lack of toxicity and a untargeted uptake, NCC has been identified as a good candidate for many bioapplications such as targeted drug carriers [3,14].

## 3. Conclusions

This review has attempted to provide a broad information about multiple features and functions of nanocrystalline cellulose (NCC). NCC is produced from a variety of cellulosic sources by chemical and mechanical methods. Most commonly is hydrolysis by acid (chemical method) and ultrasonication (mechanical method). Acid hydrolysis is a selective method, since acid only hydrolyzes amorphous regions and crystalline regions remains, while, ultrasonication is a non-selective method. It has been established that NCC obtained from composite methods (chemical and mechanical) have better dimensions and higher thermal resistance. Due to the properties of NCC such as dimension, lack

of immune response in the body, biodegradability, low cytotoxicity and hydrophilic nature caused by hydroxyl groups, increases the potential of this nanoscale material as a drug delivery excipient. Hydroxyl groups make the surface of NCC very flexible to add different chemical groups, resulting in increased drug release in tumor cells. It also makes NCC very useful in targeted drug delivery to cancer cells and increases the abruption of conjugated drugs with NCC. NCC can be influenced by various modifications in the field of biomedicine including drug carriers, diagnosis and biosensing, replacement of vascular grafts, tissue engineering, anti-bacterial, anti-viral applications, regenerative medicine, printing applications and optical application, the main findings of the present study emphasize the best NCC synthesis methods, discussing about the physical and chemical characteristic of NCC, medical applications and the role of this nanoparticle in cancer drug delivery.

## Conflicts of interest

The authors have no conflicts of interest in regard to this research or its funding.

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