Bacterial Nanocellulose -A Remarkable Polymer for Biomedical Applications: Production, Engineering, and Recent Advances and Developments

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ABSTRACT: *Bacterial NanoCellulose (BNC), a unique and promising natural polymer, due to its renewability, excellent biological features, remarkable physical properties, and special surface chemistry has received much attention for biomedical applications in recent years. There are several methods for synthesizing BNC, each with its own set of benefits and drawbacks. Modification approaches are used significantly to improve the properties of BNC or BNC-based structures for long-term and short-term biomedical applications. The fabrication of BNC-based antimicrobial materials for wound dressings, drug delivery, and hard and soft tissue regeneration is a major concern of many researchers. A wide range of biomaterials such as antibiotics, metal, and metal oxide nanoparticles are used for preparing BNC-based antimicrobial structures. In this review, we presented the main and necessary information on the key aspects of synthesis and BNC properties. Furthermore, recent literature related to the preparation and biomedical applications of BNC-based materials is reviewed. Aligned with the current trends in BNC, BNC-based biocomposites present a great field to be explored and other amazing characteristics can be expected in relation to soft and hard tissue repair, drug delivery, and other biomedical applications in the near future.*

KEYWORDS: *Antibiotics; Antimicrobial; Bacterial nanocellulose; Modification.*

INTRODUCTION

Nowadays, biopolymers such as proteins and polysaccharides, are being significantly used for producing advanced biomaterials. In a wide range of materials, Bacterial NanoCellulose (BNC) and its family members due to their unique properties such as biocompatibility, high liquid holding capacity, conformability, porosity, stability at room temperature and in wet conditions, high purity, high Young's modulus, and green processing are highly regarded as promising tools for clinical and biomedical applications. The existence of hydroxyl groups in BNC improves its compositing with other materials that create amazing structures

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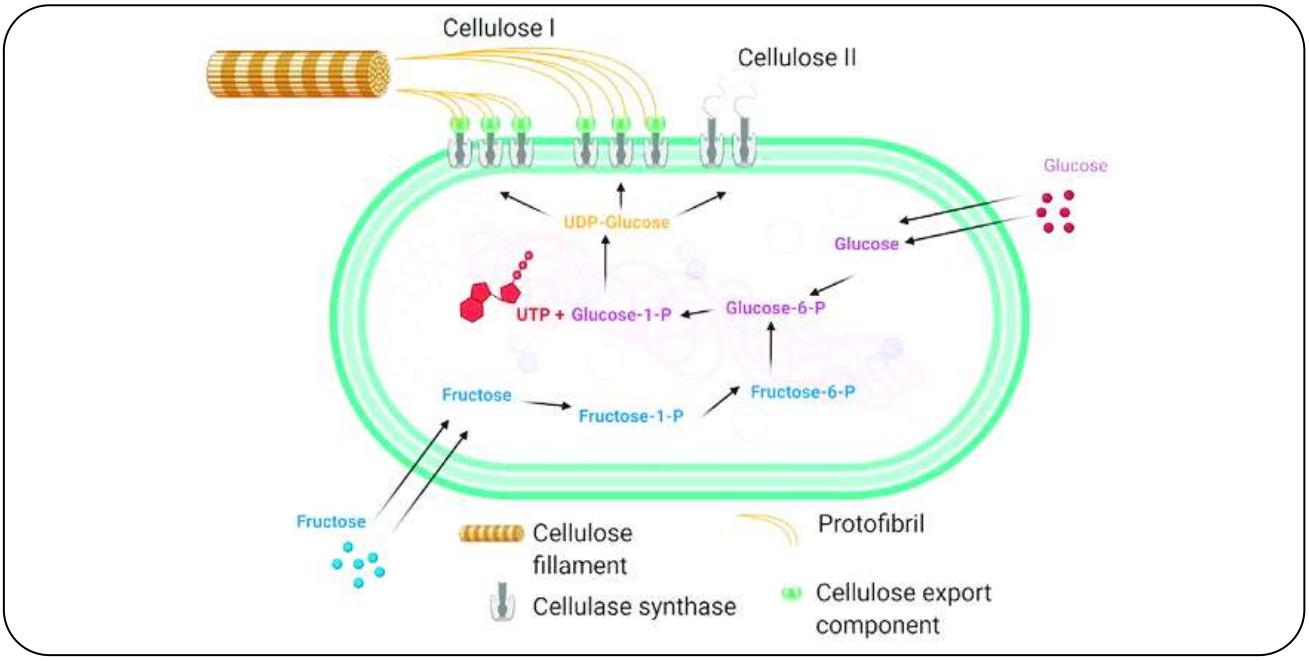


Fig.1: Schematic diagram of the biosynthesis of bacterial cellulose I and II from glucose and fructose[4].

with interesting properties such as electro-conductivity and antibacterial activity. *Acetobacter xylinum* is the best cellulose-producing bacterium that synthesizes and secretes cellulose as part of its metabolism of glucose. For BNC production, different carbon sources from the medium were used by bacteria to polymerize into β–1, 4–glucan chain, then these chains transported outside the cells via the membrane pores. Subsequently, β–1, 4–glucan chains formed subfibrils, then the subfibrils are crystallized into microfibrils, bundles and ribbons respectively. A large number of regulatory proteins and genes coding individual enzymes participate in this process [1, 2]. The Embden Meyerhof Parnas route, Krebs cycle, gluconeogenesis and phosphogluconate pathway are the most important metabolic processes for biosynthesis of BNC. The basis of the Krebs cycle is based on the oxidation of fats, proteins and acetate-derived carbohydrates while the oxidation of carbohydrate is the basis of the phosphogluconate cycle. There are four important and necessary enzymatic steps for BNC synthesis including a: conversion of glucose to glucose-6-phosphate (Glc-6-P) via phosphorylation, b: isomerization of Glc-6-P to glucose- 1-phosphate (Glc-1-P) by phosphoglucomutase, c: producing uridine diphosphate glucose from Glc-1-P by UDP-glucose pyrophosphorylase, d: polymerization of UDP-glucose into β-1, 4 glucan chains (Fig. 1)[3]. BNC can be synthesized by several bacteria and under different culture conditions that determine its wonderful physicochemical properties and morphologies. BNC synthesis methods are influenced by various factors such as selection of source, types of bacterial strains, temperature, surface area and pH, presence of other microorganisms and shape of the bioreactor. Three important and common methods for BNC synthesis are: agitated, static and bioreactor-based bacterial culturing[2]. Static culture produces gelatinous pellicles and in agitated culture system, irregular pellets suspended in culture medium are seen. The BNC produced by agitation fermentation shows low degree of polymerization, low level of crystallinity, low mechanical strength and yields, in addition, agitated culture has microbe mutations, but instead static culture needs a long culture period and high cultivation area. In the fermentation medium carbon source, phosphorus, nitrogen, sulfur, potassium and magnesium salts are necessary for synthesis process and many chemical agents affect significantly the synthesis of BNC. Fortunately, a cell-free enzyme method is used to manufacture BNC and this method can be used to produce large volumes of it. In cell-free enzyme system, BNC is produced through the anaerobic biosynthesis process with pellicles morphology and change to sheet-like structures at the air–liquid interface. In Table 1, the advantages and disadvantages of different methods are presented[2].

Table 1: Comparison of several BNC production techniques.

Production method	Advantages	Disadvantages	Description	References
Static technique	Simple method- No need for complicated tools	longer fermentation time-pellicle and reticulated slurry forms of BNC affect its yield	BNC is in the form of hydrogel sheet and is produced at the air-liquid interface.	[5]
Agitated technique	Applicable for large production volumes-Increased delivery of oxygen to bacteria-Production of BNC in various sizes and shapes	Low yield; Culture instability problems.	In this method BNC is synthesized from the particle's center and subsequently spreads outwards	[6]
Static intermittent fed-batch and repeated batch technique	Easy method- Large scale productivity	No appropriate monitoring of culturing conditions	Fresh culture mediums increase pellicle growth rate	[7,8]
Cell-free extract technique	Simple and easy method- Enhanced yield	Lack of control of synthesis process parameters	Cell lysis releases necessary enzymes to the medium	[9]
bioreactor-based technique	High yield- Efficient oxygen supply-Production of homogenous structures	Requires careful control of synthesis conditions for high volume productions	The bacteria are soaked in nutritional media and exposed to air	[10]

BNC is a pure material due to the absence of lignin and hemicellulose, therefore, it is introduced as a non-genotoxic, non-cytotoxic and biocompatible polymer. Nevertheless, it is not degraded in the human body and doesn't have suitable interaction with cells and biomolecules. To overcome these limitations, physical and chemical modification of BNC is necessary by using in situ and ex situ techniques. Changing the culture medium, carbon source and adding of other biomaterials are used for in situ modifications while chemical and physical treatment are commonly used for ex situ modifications of BNC [11, 112]. In Table 2, several modifications techniques of BNC and its properties and biomedical applications are summarized.

Important Parameters Controlling BNC Production, Properties and Biomedical Applications

There are several factors such as pH, temperature, cultivation condition and dissolved oxygen that extensively affect BC (Bacterial Cellulose) production and need to be optimized. Temperature: many studies confirmed that, a temperature range of 25 to 30 ℃ is the best for BNC production and 28 ℃ is so suitable for *Acetobacter xylinum* to produce BNC. Denaturation of the culture medium is seen at high temperatures, while cellular metabolism significantly decreases at low temperatures. In vitro studies confirmed that the optimum temperature for A. xylinum in static condition is in the range of 20-30 °C. Maximum cellulose production by *Acetobacter pasteurianus* RSV-4 and *Komagataeibacter xylinus* B-12068 was at 30 °C. It has been revealed that *A. xylinum* 0416 at 20 °C and 35 °C showed high growth rate without any lag phase. The nucleic acids and proteins of bacterial cell can significantly be denatured at high incubation temperature, even in an ideal growth medium [34]. pH: neutral and acidic environment are so ideal for BNC production. During the synthesis process, production of compounds such as gluconic acid, acetic acid and lactic acid change significantly the pH of culture medium, therefore, the best pH for the BNC synthesis is in the range of 4–6[35]. Many researches showed that, the pH of 5.50 had the best efficiency for *Acetobacter xylinum* [36, 37]. It is known that the changes in pH can affect the biochemical reactions in the culture medium. Cellulose synthesis process is an energy-dependent process and the use of glucose as the only source of energy can extensively increase the formation of gluconate, which reduces the pH of the medium and limits the BC production [38]. Culture media: the main components of culture medium for BNC production are nitrogen, in the form of casein hydrolysate and peptone and carbon in the form of glycerol, starch, fructose, maltose and xylose [39]. The necessary nutritional components such as nitrogen concentration have strong effects on the yield of BC. Each bacteria needs the specific optimal conditions for the growth and its productivity depends on significantly to these condition. Another micronutrients such as ethanol, potassium and magnesium salts and disodium phosphate play an important role in BC production, which improve cellulose yield. There are two main methods that are frequently used for BC production, namely static method and agitation method. BC produced by static method is in the form of gelatinous pellicle at the air–liquid interface. This technique needs longer cultivation time and extensive

Table 2: Modifications of BNC and its properties and biomedical applications.

Modification with…	Biomedical applications	Resulting properties	References
Glycidylmethacrylate (GMA)	Wound dressings	High resistance to the stress and strain- formation the dense thick network	[13]
Fibroblast cell line	Soft tissue engineering	Interaction between BNC and the proteins enhanced the thermal stability of the proteins and reduced the onset temperature of BNC	[14]
Iron oxide nanoparticles	Blood vessels	A structure with a Young's modulus similar to blood vessels	[15]
Chitosan	Antibacterial applications	The presence of chitosan in composite structures inhibited protein synthesis in bacteria	[16]
Gelatin	Bone tissue engineering	Modification increased mechanical strength of scaffold with the best adhesion, viability, proliferation and osteogenic differentiation of the hBMSCs	[17]
Sesamum Oil	Wound Dressing	decrease in layer wettability, considerable antibacterial property against gram-positive and -negative bacteria	[18]
Hyaluronic acid and gelatin	Stem cell therapy applications	The scaffolds showed high cell adhesion and the cells distributed within the fibers	[19]
Soy protein	Bone tissue engineering	Modification caused the scaffold became more stretchable and increased degradation rate with suitable cytocompatibility	[20]
Ethylenediamine	Manufacturing 3D bioactive scaffolds	Modified scaffolds presented high bioactivity and improved the absorption of the deposited CaO	[21]
Hyaluronic acid	Dental materials scaffolds	Modified scaffolds showed suitable interaction with calcium phosphate	[22]
Gelatin	Skin regeneration	Modified structure showed good adhesion and proliferation of human keratinocytes	[23]
Sodium alginate	Carrier system for controlled protein drug delivery	Modified composite was a promising tool for carrying hydrophilic protein-based drugs and did not exhibit any cell cytotoxicity.	[24]
Lithium chloride (LiCl), N,N-Dimethylacetamide (DMAC)	Membrane for Antibacterial and wound Healing	Modified structures promoted wound healing through accelerating the re-epithelialization	[25]
KCl/HCl solution	Dental applications	Modified BNC can be used as a wound coverage for dental treatment and appropriate antibiotic efficiency by combination with doxycycline	[26]
Water-soluble poly(ethylene glycol)	Anti-inflammatory drug delivery systems	Modified structure increased loading capacity and accelerated drug release and improved transparency supports wound inspection through the dressing	[27]
Polyhydroxyalkanoates	Bone tissue engineering	Modified scaffolds supported 3T3-L1 preadipocytes proliferation and increased in vivo osteoblast differentiation	[28]
Alginate	Localized doxorubicin release in human colorectal HT-29 cells	Modification increased surface area of scaffolds and improved drug release rate	[29]
Chitosan	Surgical meshes	Modified BNC mesh does not irritate and sensitize and does not cause hypersensitivity in the implant site, and therefore shows a low risk of provoking such reactions in humans	[30]
TEMPO(2,2,6,6-tetramethylpyperidine-1- oxyl)	Hernia repairing	The modified structure caused less inflammation and was surrounded by newly formed tissue and increased the expression of type I collagen in fibroblasts	[31]
Laser hole forming and selective oxidation	Urethral repairing	Modified BNC-containing composites with suitable mechanical strength and high cell interactions are new urethral reconstruction materials in clinical applications	[32]
HOOC-PEG-COOH-coated iron oxide nanoparticles (PEG-IONs)	Vascular tissue engineering	The modified structures presented better adhesion and proliferation of endothelial cells with high potential for vascular tissue engineering.	[33]

space for BC production, then had low productivity. In the agitated culture, the bacterial have enough O_2 and nutrients to produce BC in the forms of pellets, irregular masses, or fibrous suspensions [40]. Another important component for the synthesis of cellulose, are vitamins such as pyridoxine, nicotinic acid, biotin and p-aminobenzoic acid that must be present in the culture medium to regulate cellular metabolism and growth. Oxygen Level: O_2 plays an important role in BNC production. To achieve the highest yield of BC, O_2 must be dissolved in the media. Lack of sufficient O_2 not only impairs cellulose synthesis but also severely reduces the quality of BNC [36, 41]. BNC presents superior and unique properties, which make it so attractive for biomedical applications. It possesses the nanofibrils 3D network which provide high mechanical strength and high surface area of BNC. Cytocompatibility and interconnected porous structure cause excellent wear resistance. BNC nanofibrillar structure and its polysaccharide nature reduce the immunogenicity of it. BNC shows nonenzymatic hydrolysis in vitro, while in human body it is non-degradable due to the lack of cellulases, therefore, BNC-based structures are so suitable for long-term applications. The various strategies are used to increase BNC degradation rate including oxidation and incorporation of cellulases within it. BNC biomedical applications significantly depend on its intrinsic properties and structures, then some of these properties are not suitable for every biomedical application and should be tailored for special use. BC porous structure allows loading of different drugs and various biomolecules within it. BNC-based membranes are used to prepare dermal patches due to their high mechanical strength that provides mechanical protection to the damaged sites, providing a moist environment, decreasing pain, prevention of the allergic reactions, absorbing excess exudate and easy replacement. In vitro and in vivo studies confirmed that, various nanoparticles, drugs, biomolecules, cells and biomaterials can be incorporated into BNC-based materials. High water holding ability of BNC makes it an excellent candidate for skin tissue regeneration. It has been used to produce small-diameter vascular grafts, dental membrane, and dental cement, urethral and neural implants. In recent years acetylated BC coated with urinary bladder matrix has been used for adhesion and proliferation of retinal pigment epithelium cells. BNC is considered as a potential candidate for cornea replacement [42]. Many *in vitro* studies showed the BC could be used as a safe and biocompatible alternative to temporalis fascia with shorter surgery times and improved hearing [43].

Antibacterial Activities of BNC Composites

Antibacterial materials have been extensively used in tissue engineering and biomedical applications. As the number of antibiotic-resistant bacteria is increasing day by day, many studies about antimicrobial materials must be done to overcome the challenges of infection. In recent years, BNC has attracted a lot of attention for antibacterial applications due to its wonderful physiochemical properties. There are 4 main and basic methods for manufacturing nanocellulose-based antimicrobial materials, including antibiotic addition, surface modification, combination with nanomaterials and combination with antibacterial polymers [44]. Each of these methods are described in next sections.

Surface modification method

There are two kinds of NC (nanocellulose) with suitable antibacterial property and high biocompatibility including aldehyde-NC and quaternized NC. BC, a linear polymer glucan, is composed of glucose units and β-(1–4)-glycosidic bonds link these units. Hydroxyl groups present on cellulose surface make it easy to modify it with various functional groups such as phosphate, sulfate, amino and aldehyde. 2, 2, 6, 6-tetramethylpiperidine-1-oxyl (TEMPO)-NaBr- NaClO and $NaIO_4$ are frequently used for oxidation of BNC. The oxidation process significantly changes BNC properties including retains the crystal structure of BNC, improves its degree of crystallinity, enhances its strength and toughness and also improves BNC degradability and protein adsorption capacity. It is confirmed that, oxidized BNC in contact with water molecules, shows suitable degradability with slow mass loss during the degradation process due to its dense and crystalline structure. C–O–C bonds in molecular chains of oxidized-BNC are broken by water molecules throughout the degradation process [45, 46]. The conventional process for synthesis of TEMPO oxidized BNC is based on the immersion of BNC sheets in sodium hypochlorite/ distilled water/ TEMPO solution. The mixture should be vigorously agitated using magnetic stirrer, then ethanol is used to quench the oxidation reaction and the BNC sheets must be washed with distilled water to remove all

of the reactants [47]. Many studies confirmed that, aldehyde-NC could accelerate wound healing and improve the formation of new blood vessels. The structures also presented high biocompatibility and high cell attachment that encourage growth of epidermal cells and wound regeneration. It should be noted that, many regents and solvents which are applied in this method are toxic and can affect the final products and reduce cytocompatibility, therefore using "green" oxidants is the best way to overcome this problem. Quaternary ammonium compounds due to their biocompatibility and antibacterial properties are extensively used for grafting to BNC. Grinding method and high pressure homogenization are used for quaternization the nanofibrillated cellulose. High pressure homogenization is so attractive for producing quaternized NFC with high mechanical strength, while grinding method damages the fiber and reduces the crystallinity. BNC-containing antibacterial materials can be produced by chemical reaction between BC and aminoalkyl groups, which are lethal to *E. coli* and no toxicity to human adipose-derived mesenchymal stem cells. Electrospinning of quaternized raw materials can significantly improve mechanical strength with amazing antibacterial activities [44].

The Addition of Antibiotic to NC

In recent years, BNC-based composites are considered as promising tools for synthesizing antibacterial materials. Ceftriaxone, chloromycetin, ampicillin, and gentamycin are added to BNC to produce antibacterial materials. Clathration is the best method for controlling the release rate of antibiotics from NC-based antibacterial materials [44]. *Lemnaru et al*. prepared the solutions of bacitracin / amoxicillin in different concentrations, then immersed the freeze-dried BC specimens in them for absorption of drugs [48]. *Tamahka* prepared BC- Poly Vinyl Alcohol (PVA) - ampicillin composite hydrogel as a wound dressing. The drug molecules were loaded to composite by immersion the composite in the ampicillin solution at room temperature [49]. *Liao et al.* used poly (ε-caprolactone)/cellulose acetate/dextran/ tetracycline hydrochloride electrospun composite mats for wound healing. Dextran and tetracycline hydrochloride were added to polymers solution prior to the electrospinning, then the composite solution was placed into a syringe for electrospinning [50]. *Kui Ho et al.* produced cellulose acetate nanoparticles via the nanoprecipitation process and loaded Penicillin G into them by immersion the nanoparticles in phosphate buffer saline (PBS, pH 7.4) and incubation for 72 h [51]. *Adepu et al*. used the polyethylene glycol 2000 for modification of BC and investigated modified BC properties and drug loading efficiency and drug release kinetics. Solvent evaporation technique was used for drug loading, in which the BC sheets were soaked in diclofenac sodium stock solution for 8 h [52]. *Jantarat et al.* prepared BC-Molecularly Imprinted Polymer (MIP) composites for sustained-release of quercetin. Molecularly imprinted BC was fabricated through the phase inversion method. BC was put in NaOH+urea solution and placed in the freezer for complete dissolution of BC. Quercetin was dissolved in methanol and added to BC solution to obtain a homogeneous mixture [53]. *Shao et al*. tetracycline hydrochloride was loaded into BC. BC was cut into small pieces and immersed in aqueous tetracycline hydrochloride solution with different concentration and stirred at dark condition. The samples were rinsed with de-ionized water and freeze-dried at -40 ºC for further studies [54]. In Fig. 2, the commonly used antibiotics are presented. In Table 3, all kinds of antibiotics which are used for preparing BNC-based antibacterial structures are shown.

Combination with Nanomaterials

In recent years, nanotechnology has a significant effects on human life. Nanoparticles have unique characteristics and can be used for many aims. The used of them in microbiology and biomedical applications due to their excellent biological, physical and chemical properties has been developed. Various types of metal and metal oxide nanoparticles show antimicrobial activity against both Gram-positive and Gram-negative bacteria and are extensively used for production of BNC-based antibacterial materials. Silver and gold nanoparticles due to their low-toxicity, high surface area and high surface modification ability are so attractive for preparing antibacterial structures. The performance of gold nanoparticles is similar to applying pressure to a balloon, with areas of the cell wall stretching and deforming at different points until the bacteria exploded. It has been confirmed that, spherical nanoparticles damaged the bacteria more effectively and rapidly. Silver nanoparticles aggregates are very common, which can change their antimicrobial activity, but the combination of these

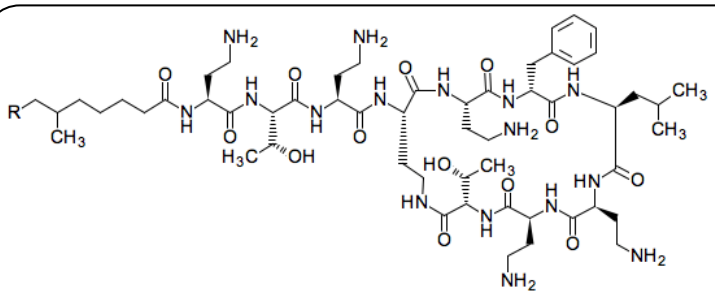
Table 3: Common used antibiotics for producing BNC-based antimicrobial materials.

Antibiotics	Bacterial species	References
Ceftriaxone	S. aureus	[55]
Amoxicillin	Staphylococcus aureus - Escherichia coli	[48]
Ceftriaxone	Staphylococcus aureus	[56]
Dexpanthenol	Staphylococcus aureus	[57]
Gentamycin	Escherichia coli- Klebsiella pneumonia- Staphylococcus aureus- Streptococcus mutans	[58]
Povidone iodine -Polihexanide	Staphylococcus aureus	[59]
Gentamicin	E. coli - S. aureus	[60]
Ampicillin	S. aureus- P. aeruginosa- E. coli- E. faecalis	[61]
Amoxicillin	Fungus- Gram-negative- Gram-positive bacteria	[62]
Tetracycline hydrochloride	Escherichia. coli- Staphylococcus aureus- Bacillus subtilis- Candida albicans	[54]

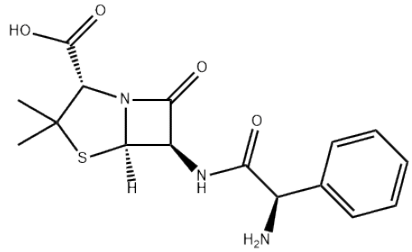
nanoparticles with BNC significantly improves their dispersion pattern. Furthermore, silver ions will block the bacterial respiratory system and thereby destroy the energy production of the cell. In the end, the bacterial cell membrane will burst, and the bacteria will be destroyed [63]. Many studies have examined the antibacterial effect of metal oxides such as titanium dioxide (TiO_2), copper oxide (CuO), zinc oxide (ZnO), and magnesium oxide (MgO). Important parameters such as crystal structure, shape, and size significantly affect antimicrobial property of TiO_2. Illumination of TiO_2 produces highly oxidizing free radicals that oxidize and inactivate microbes. TiO_2-coated surfaces have shown lowered bacterial load. The radicals do not induce antimicrobial resistance, an important cause of concern in the medical context. TiO_2 is non-toxic and chemically stable and is currently used for implants. CuO nanoparticles interact closely and strongly with bacterial membranes and damage their DNA and vital enzymes. ZnO has strong and effective antimicrobial properties against a wide range of pathogens. ZnO and MgO nanoparticles can cross microbe membranes, disrupt metabolic pathways, change membrane shape, deactivate vital enzymes and proteins, and increase oxidative stress in bacterial cells. The photocatalytic property of ZnO nanoparticles have been confirmed in recent years and they are more biocompatible than TiO_2. Photocatalytic reactive oxygen species (ROS) production is the main responsible for killing the bacteria. UV light increases ROS production, and these productions damage lipids, proteins, vital enzymes and nucleic acids [64]. In Fig.3, several useful antimicrobial nanoparticles and their mechanisms are presented.

Combination with Antibacterial Polymers

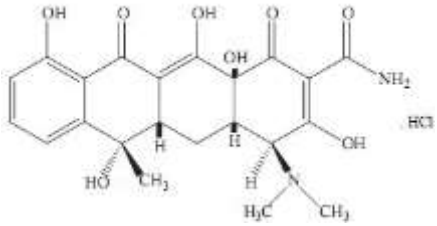
Natural polymers with inherent antimicrobial activity are attractive candidates for use in biomedical applications. The cationic charge and hydrophobicity of these polymers play essential roles in their antibacterial activity. Cationic polymers combat bacteria via electrostatic, in which cationic charged antimicrobial polymers bond to the anionic charged bacterial membrane, disrupt the membrane, and ultimately prevent cell growth. Another hypothesis is that antimicrobial polymers release low molecular weight antimicrobial agents that can bind to the membrane proteins and penetrate the cell wall. Antimicrobial agents interact with cell DNA and affect DNA transcription and mRNA synthesis [66]. Chitosan (CS) is the most famous and applicable natural antimicrobial polymer against an extensive variety of microorganisms. CS, an aminopolysaccharide biopolymer, has a unique chemical structure as a linear polycation with a high charge density, reactive hydroxyl and amino groups as well as extensive hydrogen bonding. t displays excellent biocompatibility, physical stability and processability. The most prevalent proposed antibacterial activity of CS is by binding to the negatively charged bacterial cell wall causing disruption of the cell, thus altering the membrane permeability, followed by attachment to DNA causing inhibition of DNA replication and subsequently cell death. BNC-CS composites showed amazing features such as ultrafine nanofiber network,



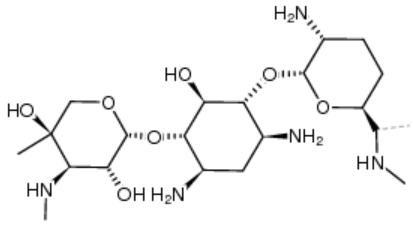
Polymyxin B



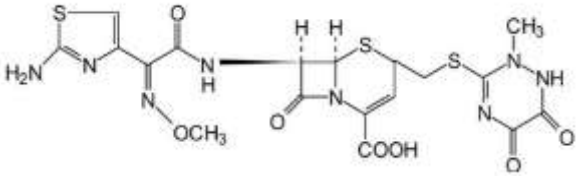
Ampicillin



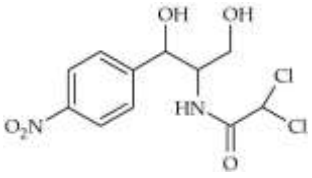
Tetracycline hydrochloride



Gentamicin



Ceftriaxone



Chloromycetin

Fig .2: The structure of antibiotics in NC-based antibacterial materials [39].

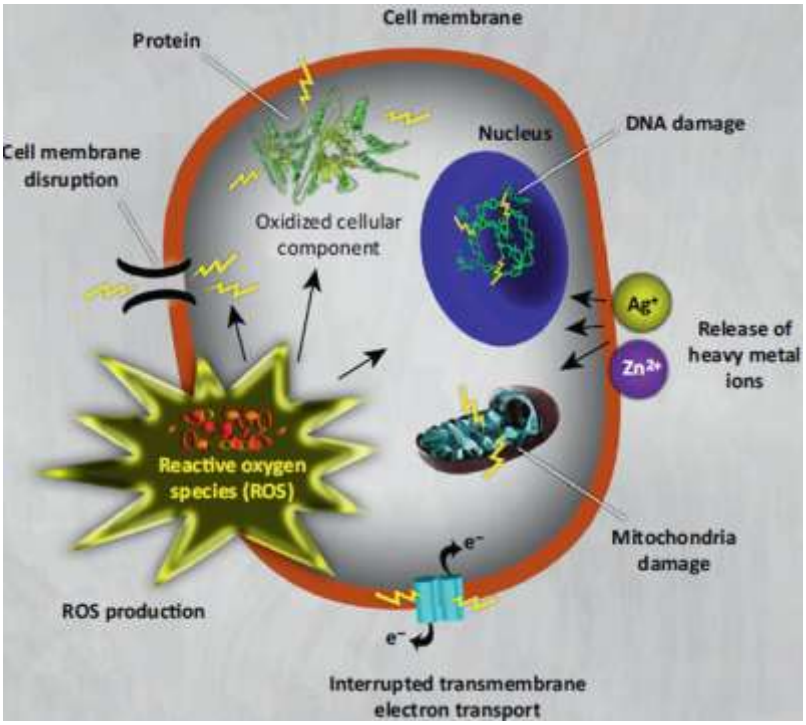


Fig. 3: Schematic presentations of the antimicrobial mechanisms of several nanoparticle [52].

ability to hold large volumes of water, high tensile strength and elastic modulus. The cumulative release rate of CS from the composites depend on its molecular weight (Mw) and pH of solution. Many studies confirmed that with the increasing of CS Mw, the antibacterial activity increases accordingly [67]. Polylysine has a high positive charge density, enabling it to form soluble complexes with negatively charged macromolecules. Polylysine is a cationic polymer that binds heavily to negatively charged DNA, therefore, it can easily damage microbial cell membrane and inhibit their proliferation. BC- Polylysine composites showed excellent antimicrobial activity against both *E. coli* and *S. aureus* [68].

BNC-based Structures Biocompatibility for Biomedical Applications

Biocompatibility is a general term describing the property of a material being compatible with living tissue. Biocompatible materials do not produce a toxic or immunological response when exposed to the body or bodily fluids. In recent years, many studies have examined the biocompatibility of BNC [44]. Table 4, shows recent studies about BNC toxicity and their conclusions for biomedical applications.

BNC, A versatile polymer: From hard tissue Engineering to complex soft tissues regeneration

In recent years polysaccharides-based materials are used extensively in tissue engineering, usually in hard tissue regeneration. BNC-based 3D scaffolds are so attractive over other biomaterials due to their excellent biocompatibility, suitable mechanical properties, high surface area and renewable nature. The nanofibrous network of BNC shows wonderful effects on cell behaviors such as attachment, migration, proliferation and differentiation. BNC is insoluble in aqueous media and has excellent thermal stability, furthermore, it cannot be biodegraded in-vivo due to the lack of cellulase enzymes in the human body. BNC is not inherently antibacterial, but the adding external antimicrobial agents can cause this property in it. BNC can be used as ideal delivery of recombinant human BMP-2 (rhBMP-2) for bone tissue repairing. Further, BNC scaffolds containing BMP-2 a nd cells present considerably improved bone matrix secretion [89]. In a study, BNC scaffold loaded with fisetin and bone marrow MSCs showed high cell viability and improved differentiation of bone marrow MSCs into the osteoblasts and also increased the expression of osteocalcin and osteopontin genes in the cells [90].

Table 4: Recent studies about toxicology experiments and conclusions for BNC.

Toxicological experiment	Conclusion for biomedical applications	References
L929 cells	No cytotoxic-Auricular cartilage regeneration	[69]
RSC96 cells	Good biocompatibility- Scaffold material for neural tissue engineering	[70]
Human dermal fibroblast cell	Cell proliferation promoted-Wound dressing	[71]
Pig iliac endothelial cells (PIECs)	Cell proliferation promoted-BNC conduits for blood vessel applications	[72]
Hemolysis assay	Highly hemocompatible-Soft tissue implants	[73]
L929 cells	High biocompatibility-Non viral gene delivery system	[74]
M3TCT3-E1 pre-osteoblast cells	High biocompatibility and Cell proliferation promoted	[75]
Schwann cells	Good biocompatibility	[76]
Shell-less hen's egg model	Good biocompatibility- Gene activated matrix	[77]
Shell-less hen's egg model	Excellent biocompatibility- Drug delivery system	[78]
Chondrocytes	Excellent biocompatibility- Cartilage tissue 3D printing	[79]
Human monocyte/macrophage cell line, THP-1	Good biocompatibility- Implantable meshes	[80]
Rat basophilic leukemia RBL-2H3 cell line	Cell proliferation promoted- Mesh for biomedical applications	[81]
Chondrocyte	Excellent biocompatibility- Cartilage implant	[82]
Primary human fibroblasts	Promote wound repair- Wound healing	[83]
Human Umbilical Vein Endothelial Cells	High biocompatibility -Scaffolds with a complex vascular mimetic lumen structure	[84]
Fibromuscular cells	Promote wound repair-Alternative patch material in congenital heart disease	[85]
Mesenchymal stem cells (MSCs)	Excellent adhesion ability-Tissue engineering	[86]
Whole blood clotting assay	Highly hemocompatible- Hemostatic dressings	[87]
Human embryonic stem cell-derived limbal stem cells	Cell proliferation promoted- Ocular surface pathologies	[88]

BNC is also used frequently in dental field. It can be used for Guided Tissue Regeneration (GTR) technique at periodontal diseases treatment. Many studies showed that BNC membranes were successfully used in association with bone-integrated implants. BNC-based composites such as BNC- alginate structure are applied as a temporary dressing of oral surgical flaps due to excellent potential of BNC for use in the oral cavity to cover surgical wounds [91]. Fig.4, shows some examples of the BNC-based temporary wound dressing. In endodontics field, BNC is an amazing biomaterial for dental root canal treatment since don't present harmful effects, and also in wet conditions, it shows a high absorption rate without deformation. It is an amazing candidate for reinforcing other biomaterials such as dental cements due to decreasing the setting time and increasing the mechanical strength of cement. It can be concluded that BNC due to its unique properties has attracted much attention for biomedical and tissue engineering applications. Dental applications of BNC have certain complexities, since several its features such as elasticity, mechanical stability, and hemostatic, easy handling during surgery and acting as a barrier against microorganisms, directly affect the oral mucosa and periodontal tissue regeneration process. By considering the satisfactory results of many studies conducted to date, it can be hoped that unpredictable properties for these materials will appear in the future [91]. As reviewed and discussed in this section, many BNC-based biomaterials for bone tissue engineering/dental and oral fields are presented in Table 5.

Lately, in soft tissue field, the use of BNC has attracted much attention due to its satisfactory performance. It can be used as both matrix and reinforcement material. Many studies described BNC applications for cardiovascular, nervous and urinary tissue regeneration. This polymer due to its amazing properties such as mechanical and physical

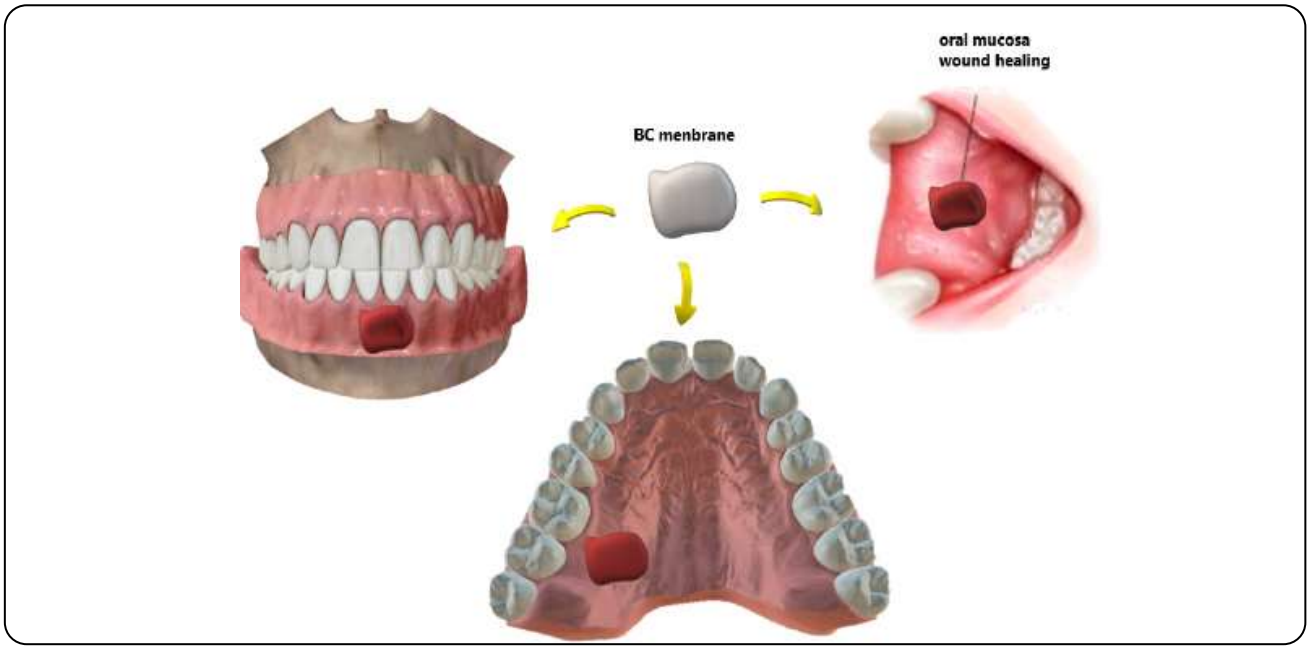


Fig.4: BNC-based dressings cover many types of wounds in the oral mucosa [77].

stability, high purity and biocompatibility is extensively used as wound dressing material, artificial skin and blood vessels. BNC as wound dressing structure, promotes autolytic debridement and accelerates granulation, furthermore, it can regulate the moisture content of the wound area, sometimes by absorbing moisture from the wound area and sometimes by releasing fluid to the same area. Despite many benefits of BNC, there are serious and challenging limitations to the application of wound healing such as absence of antibacterial property, stress bearing capability and optical transparency. To overcome these challenges, compositing with other materials is very efficient. In normal condition, blood vessels are responsible for blood circulation throughout the body and all tissues. When pathogenic agents damage the vessels, it is necessary to replacement with artificial blood vessels. BNC are frequently used as a synthetic graft and it must be tested for resistance to high blood pressure. In vivo studies confirmed that BNC graft could integrated with the surrounding tissue and no infection was found at the damaged site [115]. This polymer is suitable for small diameter vessels, but side effects should be removed [81]. BNC is also an important candidate for nerve tissue regeneration due to its versatile surface chemistry and physiomechanical properties. The combination of this polymer with conductive structures can accelerate neural tissue repairing [116]. Bladder cancer is another soft tissue that needs new biomaterials for urinary tissue reconstruction, BNC can provide good growth conditions for urine derived stem cell and enhance new tissue formation [117]. Corneal injuries and ulcers need keratoplasty to restore eye integrity, BNC has high potential to treat eye disease. Its high mechanical strength, water holding ability, good transparency, permeability to liquids and gases make it an appropriate material for contact lenses [118]. BNC is used for prosthetic meshes in abdominal wall defects due to its strain and elasticity [119]. In summary, in vitro and in vivo studies confirmed that BC had high potential for biomedical and tissue engineering applications. Although results described BNC as a substitute for hard and soft tissues, more clinical studies are required to gain the structures that mimic the properties and performance of native tissues.

CONCLUSION AND FUTUR PROSPECTS

BC is an organic compound produced by specific types of Gram-negative and Gram-positive bacteria. BC production depends on many factors such as the growth medium, environmental conditions and the formation of byproducts. In recent years, BC and its family members have been widely used in medicine and engineering. Genetic modification of it, is essential for stable and

Table 5: Potential BNC-based biomaterials for dental and bone tissue engineering.

Composition	Potential use	Bone tissue engineering	Dental and oral treatments	BC advantages	References
BC	Barrier membrane	-	Periodontal treatment	Improved cell attachment and proliferation with no chronic inflammatory response	[92]
BC	Scaffold	-	Dental pulp tissue treatment	Enhanced the hardening processes of cement-Induced mineralized barrier and apical closure	[93]
BC	Surgical suture	-	Dental surgery	Increased mechanical strength and promoted cell proliferation-BC-based yarn significantly promoted wound healing without obvious adverse effects	[94]
BNC	Barrier membrane	Guided bone regeneration	-	Low biocompatibility- large amount of mature connective tissue in the defect sites	[95]
BNC	Barrier membrane	-	Regenerative endodontics	Membranes showed good biocompatibility and inhibited biofilm formation	[96]
BNC	Membrane	-	Periodontal tissue regeneration	Promoted cell proliferation and enhanced formation of mineralized nodules	[97]
Cellulose nanocrystals	Nanocomposite	-	Dental adhesive	Showed a slight improvement of flexural strength	[98]
Nanocellulose	Composite	-	Flowable dental composites	Increased the compressive strength of the flowable dental composite and permitted their utilization in stressed areas	[99]
BNC	Patches	-	Aphthous Stomatitis Treatment	patches were non-cytotoxic and enhanced drug delivery- BC improved thermal stability of the patches- composite patches retained their mechanical integrity after water absorption	[100]
Biocellulose nanowhisker	Composite	-	Cement composites for endodontic applications	Improved the hardening processes of cement and improved the hydrosilicates formation	[101]
BNC	Film	-	Wound dressing	Improved mechanical qualities, and excellent cell adhesion and proliferation	[102]
BNC	Membrane	Guided bone regeneration	-	Increased the amount of new bone formation and fibrous connective tissues	[103]
BNC	Nanocomposite	Bone regeneration	-	Promoted the formation of bone like apatite and stimulated the early development of the osteoblastic phenotype	[104]
BNC	Hybrid materials	Bone repair	-	Increased the release of Sr^{2+} and modulated bone repair	[105]
BNC	Membrane	Bone tissue regeneration	-	Enhanced osteogenic differentiation of cells and increased osteogenic gene expression	[106]
BNC	Biphasic calcium phosphate scaffold	Bone tissue regeneration	-	Improved bone regeneration, cell attachment and proliferation and increased the osteoblastic gene expression	[107]
BNC	Hydrogel	Bone tissue regeneration	-	hydrolysis with phosphoric acid modified the pattern of X-ray and decreased the crystallinity of cellulose	[108]
BNC	Gel	Bone regeneration	-	Improved compression modulus and stimulated calvaria regeneration	[109]
BNC	Nanocomposite	Bone regeneration	-	Improved mechanical properties	[110]
BNC	Nanocomposite scaffolds	Bone repair	-	Regulated the nucleation and growth of nano hydroxyapatite- promoted the proliferation of MC3T3-E1cells-promoted the formation of bone tissue in rat skull defect model	[111]
BNC	Composite scaffold	Bone support materials	-	Enhanced in vitro bioactivity and cell proliferation and increased alkaline phosphatase (ALP) activity, and osteogenic-related gene expression	[112]
BNC	Composite scaffold	Osteogenic differentiation of human mesenchymal stem cells	-	Improved physical stability of scaffold and osteogenic differentiation of cells	[113]
BNC	Composite film	Bone regeneration	-	Improved thermo-mechanical stability of scaffold and increased deposition of Ca–P mineral on its surface	[114]

cost-effective applications. BNC has more remarkable features as compared to other materials, such as biocompatibility, good physical and mechanical properties, thermal stability and high crystallinity, therefore, it is a promising tool for biomedical and tissue engineering applications. There are several challenges about it, such as its insolubility in water and several other solvents, which limit its use in designing scaffolds for soft and hard tissues engineering. This problem can be solved by applying modified BNC. Physical and chemical modifications or combination with other biomaterials can significantly enhance BC properties for a wide range of applications such as wound healing process, drugs and biomolecules delivery systems, implants and grafts for different parts of human body. In many cases, low degradation rate of BNC-based structures is useful for tissue regeneration, but low solubility, low permeability and low bioactivity of it is serious challenges to use in biomedical area. It is necessary to apply the green method with low-energy and low cost for BNC. The antibacterial activity of BC-based structures improves by adding antibiotics including ceftriaxone, tetracycline hydrochloride, amoxicillin and etc. The overuse of antibiotics significantly causes resistance to antibiotics, then reduces the antimicrobial activity of BC. To overcome these challenges new antibacterial agents with strong effects and high durability have been developed. Metal and metal oxide nanoparticles, bioactive substances, natural and synthetic polymers and nanosilicates can be easily compounded with BC to obtain antibacterial property. BNC-antibacterial materials composites have high potential to prevent infection and accelerate wound healing, but there are some serious problems such as metal and metal oxide toxicity, environmental pollution and uncontrolled release of natural compounds should be answered in biomedical applications. BNC is a useful and appropriate biomaterial used as artificial skin, implants, and artificial blood vessels, wound dressing, soft and hard tissue scaffolds. Many in vitro and in vivo studies are needed to investigate the low-cost method for BNC synthesis that can use waste materials for feed-stock. The supplementation of culture medium with active materials can modulate the intrinsic features of BNC to obtain the excellent biological properties. Overall, the main and important goals of BNC-based studies are the fabrication of BNC-based smart composites, improvement its properties, reducing production costs and designing the suitable technique for industrial-scale production of it.

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