Advances in Bioresearch
Adv. Biores., Vol 14 (4) July 2023: 283-306

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.14.4.283306

Advances in Bioresearch

REVIEW ARTICLE

Biomedical Applications and Future Prospects For Bacterial Cellulose-Based Hydrogel Composites along with Nanoparticles

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ABSTRACT
*Unlike plant cellulose, which is derived from plants, microbial cellulose is synthesized by a wide range of microorganisms; it is an exopolysaccharide produced by bacteria with distinct structural and mechanical properties. In contrast to synthetic polymers, bacterial cellulose (BC) is a bio-based material that benefits from the unique qualities of a natural polymer. BC has been considered as a promising candidate for a wide range of biomedical uses due to its lack of toxicity, high purity, and biocompatibility. Nanocomposites made from this substance are generally regarded as being environmentally friendly. Because of bio-nanocomposites (made of inorganic and solid biopolymers) are widely used, researchers are focusing on them. In this review, we briefly discuss the structure, classification, biosynthesis, and functional properties of exopolysaccharides of microbial origin, with a particular emphasis on BC. We also discuss some of the nanoparticles, BC-based bionanocomposite, their biomedical appli*cations with some of their recent advancements, and future guidelines for enhancement of BC.
Keywords: *Bacterial cellulose, BC-based bionanocomposite*, *biomedical appli*cations

Received 17.04.2023 Revised 20.06.2023 Accepted 25.07.2023

How to cite this article:
Haseena Muhammad, Najla Ali Alburae, Salah E. M. Abo-Aba, Biomedical Applications and Future Prospects for Bacterial Cellulose-Based Hydrogel Composites Along with Nanoparticles Adv. Biores. Vol 14 [4] July 2023. 283-306

INTRODUCTION

Cellulose is typically harvested from plants or derived from plant byproducts. It is the cheapest, most abundant, and most accessible carbohydrate polymer on the planet [1]. Plant cell walls contain cellulose as a structural component, bound to other polymers such as lignin, pectin, hemicellulose, etc. [2]. Chemical processing with acids and harsh alkalis is a lengthy but necessary step in obtaining a pure form of cellulose from this polymer [3]. Due to an increase in demand for extracting plant cellulose, there is a rise in the consumption of wood as a raw material that leads to deforestation and also causes environmental issues globally [4].

Although it is challenging to isolate cellulose from plants, many different kinds of microorganisms (including fungi, algae, and bacteria) are capable of producing it [5]. Polysaccharides are basically a type of sugar found in microorganisms. Polysaccharides are long chains of carbohydrate molecules, and they are primarily composed of monosaccharide units [6]. Exopolysaccharides (EPs) are another common form of glycans, and they are found in the cell wall (EPS). Primary metabolites include EPS from cell wall biopolymers, and secondary metabolites include capsular biopolymers from bacteria [7]. Fiberized cellulose is synthesized and secreted by a wide variety of bacterial species. Similarities between these species and *Acetobacter xylinum* have been found [2].

Currently, humans started paying more attention to bacterial cellulose (BC), because it is a sustainable polymeric substance with a promising future [8]. To a large extent, bacterial cellulose, a naturally occurring polymer, can be attributed to the fact that it is the most common polysaccharide and organic substance on

Earth [6]. In 1988, while researching *Acetobacter xylinum* fermentation, *A.J. Brown* published the first report on this biomaterial, which confirmed the development of an unbranched pellicle [9]. He determined that the chemical composition of this structure is identical to that of cellulose found in plants. It consists of glucose units with the formula $(C_6H_{10}O_5)$. A large homopolymer chain of disaccharide or dimer of any hydro-D-glucopyranose unit (cellobioses) connected by β *(1- 4)* glycosidic linkages [10]. BC also features a unique nanostructure, high mechanical strength [11], high water retention [2], and a high degree of crystallinity [12]. Due to its singular structure, which consists entirely of glucose monomers, BC possesses all these characteristics. BC is still biocompatible despite its higher water content.

However, biomechanics, bioactivity, and biocompatibility are the main constituents that will be kept in mind when considering any biomaterial for therapeutic use. As expected, cellulose has all these characteristics. To improve clinical outcomes, BC has seen forextensive use in the pharmaceutical and biomedical industries. BC is used in a wide variety of contexts because of its special characteristics, such as biodegradability, non-toxicity, biocompatibility, and hydrophilicity, and because of nanocellulose in modified forms or integration with nanoparticles [13]. BC is commonly understood to be a natural hydrogel. For the most part, a hydrogel is considered to be a three-dimensional polymer network that has been swollen by a very large amount of solvent. Natural hydrogel is being phased out in favour of synthetic hydrogel because of the latter's superior durability. The high water-absorption capacity of synthetic hydrogels has also been demonstrated [14]. However, BC hydrogel can absorb water at a rate of 99% of its weight due to its amorphous structure. BC's 3-D network structure gives it unique properties like water retention and mechanical strength, which make it a suitable platform for the renewal of various tissues [15]. When it comes to wound dressings for trauma patients, an antimicrobial BC dry film is crucial because it absorbs fluid from tissue and blood from the wounded area and speeds up the wound healing process [16]. As cellulose-based hydrogels are biodegradable and nontoxic, they are therefore used in a wide variety of biomedical applications, i.e. controllable drug delivery systems. Moreover, BC-based hydrogels become more effective when loaded with nanoparticles.

In consideration of that, nanoparticles (10-100 nm; 1 m) are utilized in a wide variety of biomedical functions. In recent years, there has been a meteoric rise in the use of nano-systems in a wide range of biomedical settings. Nanoparticles like iron oxide have improved magnetic, biological, and chemical properties. Their chemical stability, nontoxicity, and biocompatibility make them ideal. As a result of its unique properties, the nanoparticle can be put to use in a variety of biomedical contexts, including drug delivery, gene delivery, and hyperthermia [17]. The potential of BC in nano-composites has only recently been investigated. It holds great potential as a green nano-composites building block. In order to take advantage of the magnetic, chemical, electrical, and optical properties of inorganic nanoparticles, several researchers have used BC as a starting point for designing hybrid inorganic-organic composites that combine the best features of both materials [18]. An increasing number of industries and academic institutions are turning to bio-nanocomposites as a viable alternative because of their versatility in potential applications.

Exopolysaccharides of microbial origin will be briefly summarized in the present study. Structure, classification, biosynthesis, and functional properties are all used as criteria for awarding the various microbial biopolymers. The structure, biosynthesis, properties, and methods of characterization of BC are the primary focus of this review. A few examples of nanoparticles, how they are categorized, and how they are synthesized in living organisms are also discussed. Bio-nanocomposites based on BC are discussed, along with their potential medical uses. The most recent developments in the synthesis of composites from various materials are highlighted. Finally, it discusses the potential for forthcoming guidelines for the enhancement of BC production and synthesis of BC-based composites, functionalization, and innovative applications in medical fields.

Exopolysaccharides

Many bacteria produce polysaccharides, which are polymers that are both water-soluble and microbial in origin [19]. It is generally agreed that exopolysaccharides (EPS) are a form of polysaccharide synthesized by microorganisms within their cells. The majority of organisms aid their healing by secreting EPS into the extracellular space [20]. While trying to classify the high-molecular-weight carbohydrate polymers, *Sutherland* coined the term EPS in 1972 [21]. These polymers are released into the environment by microorganisms. Since 40–95% of these polymers are made up of polysaccharides, we refer to them as "extracellular polysaccharides" or "EPS" [22]. Exopolysaccharide (EPS) is a type of molecule found in microbial cells that forms a layer outside the cell membrane and is characterized by very long molecular chains of sugar units. These long chains of sugar units have a molecular mass of around $0.5–2.0x10_6$ *Da* [23].

Bacteria, fungi, and yeast are just some of the microorganisms that can produce EPS [24]. Biodegradable, biocompatible, and able to form associations with many different types of macromolecules (including lipids, nucleic acids, and proteins), microbial EPS is preferable to synthetic polymers for many reasons [25]. It is produced and released into the extracellular environment through a plethora of mechanisms. EPS may excrete soluble or insoluble polymers as a defense mechanism against various environmental stresses, such as PH damage caused by UV light, osmotic pressures, defense against oxidants, and uptake of heavy metals. These polymers are also able to with-stand the extreme environmental conditions brought on by the formation of biofilms on the surface [26]. Biofilms are communities of microorganisms that are tightly interconnected [27]. Different microbial cell biofilms have increased resistance to disinfectants and nutrient depletion. In comparison to distinct microbial cells, it also shows enhanced opposition in regard to antibiotics and the influence of oxygen reactive by-products such as polysaccharide hydrogen peroxide and hydrogen radicals [28].

Generally, polysaccharide biosynthesis typically occurs in the later stages of microbial development. The three main categories of polysaccharides are lipopolysaccharides, cytosolic polysaccharides, and exopolysaccharides (EPSs), and their classification is based on where they are located in a microorganism's cell [29]. Homopolysaccharides and heteropolysaccharides are two subcategories of EPS. Homopolysaccharides like levan and dextran are constructed from only one type of monosaccharide. Xanthan and Magellan are two examples of the monosaccharides that make up heteropolysaccharides. Typically, heteropolysaccharides are produced in the form of repeating unit [30]. A large number of EPSs are formed from heteropolysaccharides and usually possess complex structures Regarding their applications, for a long time, polysaccharides synthesized by seaweed, i.e., alginate and agar, and plants, i.e., pectin, and starch have been used in the medical field, agricultural field, food industries, and also in research laboratories. In several scientific areas, the structure and properties of microbial EPS are gaining attention as a theme of research, specifically focusing on its use in the optimization of synthesis processes [31]. However, regardless of the vast population of EPS along with their physiochemical properties that are industrially very capable, in Europe and the United States, only two EPSs are approved for use in the food industry as additives: gellan and xanthan [32]. However, there are also some other microbial polysaccharides like levan, pullulan, dextran, and elsinan. These microbial polysaccharides have important organizational or structural characteristics that have potential applications in the biomedical, pharmaceutical, and food industries [7]. These microbial polysaccharides have important organizational or structural characteristics that have potential applications in the biomedical, pharmaceutical, and food industries [7].

Biosynthesis

The three main steps in EPS biosynthesis are (i) the incorporation of a carbon substrate, (ii) the cellular production of polysaccharides, and (iii) the extracellular exudation of the polysaccharides by the cell. Amino acids are used as nitrogen sources, sugars as a source of energy, and ammonium as a salt source by microorganisms that are involved in the synthesis of exopolysaccharides [33]. Microorganism's polysaccharide synthesis is affected by the nitrogen-to-carbon ratio in their surroundings; the *C/N* ratio of *10:1* is thought to provide the greatest scope for exopolysaccharide synthesis [34]. Currently, in bacterial species for the biosynthesis of exopolysaccharides, four basic mechanisms have been known: the Wzx/Wzy- dependent pathway, the ATP-binding cassette (ABC) transporter dependent pathway, the synthase-dependent pathway, and extracellular synthesis by using a single sucrase protein.

Wzx/Wzy Dependent Pathway

In the Wzx/Wzy dependent pathway, initially for the formation of phosphate linkage, the capsular polysaccharides, and lipopolysaccharides O-antigen link a nucleotide activated sugar such as UDP-Glc to a membrane-associated lipid carrier (undecaprenyl phosphate), i.e., E. coli group. Sequentially, higher sugar units are linked by Glucosyl-transferases for the generation of repeating units. Around the cytoplasmic membrane, repeat units are transferred by Wzx flippase. Through the action of Wxy protein, the translocated oligosaccharide units get polymerized into polysaccharides before being transported to the surface of the cell. Assembling of Polysaccharides through such pathway owns more diverse pattern of sugar and are classified as hetero exopolysaccharides [28].

ATP- Binding Cassette (ABC) Transporter Dependent Pathway

The synthase-dependent pathway is another pathway used in microbial cells for the biosynthesis of exopolysaccharide. In this pathway, an envelope-spanning multiprotein complex glycosyl-transferase has simultaneous polymer development and translocation through the inner membrane with the help of a single synthase protein, which is its subunit. It makes complete homopolymer strands on all sides of the cell membranes and cell wall which do not require any strategy like Wzx protein (flippase) for monomeric repeated units translocation [35].

Sucrose Mediate or Extracellular Synthesis Pathway

The sucrose mediates or extracellular synthesis pathway is the final pathway that initially converts disaccharide molecules into monosaccharides. Afterward, it is transported into the developing polysaccharide chain in the extracellular environment via a synthase-dependent pathway that results in a polymerization reaction [28]. Such a kind of synthesis has no dependence on central cellular carbon metabolism, having narrow structural changes. Glycan sucrase catalyzes the transportation of monosaccharides to an acceptor molecule. Due to the hydrolysis of sugar, energy is released that is used for the catalytic of glycosyl residue on developing homopolysaccharide [36].

Cellulose from Bacteria

Numerous microorganisms play crucial roles in mitigating environmental stress and facilitating environmentally friendly procedures. Despite the many ways in which microorganisms contribute to environmental sustainability, they are often poorly represented in scientific discourse. Eukaryotes and prokaryotes alike are capable of synthesizing biopolymers. Enzymes are responsible for their production, and they are often secreted as compounds outside of cells [37]. Cellulose can be synthesized by microorganisms and is considered one of the most profuse, ubiquitous, and mostly used natural polymers. It is also synthesized by various marine animals in laboratories, plants, and cell-free systems [38]. Cellulose is one of the most abundant, ubiquitous, and commonly used natural polymers, and it is synthesized by microorganisms. Several marine organisms, plants, and cell-free systems have been shown to synthesize it in controlled environments [38].

A. J Brown discovered BC produced from *Acetobacter Xylinum* for the first time as an extracellular gelatinous fiber [39]. Bacterial cellulose (BC) is considered a homopolysaccharide, formed by linear monosaccharide chains of β-D-glucose connected by a β (1 → 4) bond [40]. The non-pathogenic gram-negative bacterial strains that synthesize BC extracellularly are *Aerobacter*, *Achromobacter*, *Azotobacter*, *Alcaligenes Arhizobium*, *Pseudomonas, Sarcina, Rhodobactera, and Dickeya* [41]. While *Komagataeibacter xylinus* was initially identified as Acetobacter *xylinum*, which is a member of the *Glucobnacetoacter xylinus* species. It was popular for its ability for the commercial-scale production of BC [42]. BC that has been synthesized from this strain has application in many areas, i.e. packaging material and food. However, BC is used in many other fields recently, like biomedical, and tissue engineering. In terms of the procedure for synthesis, the terminal complexes (TCs) receive the excreted proto fibrils from microbial cells which synthesize β-1, 4-glucan chains intracellular. TCs are positioned outside the barrier of microbial cells [43]. The β-1, 4-glucan chains crystalize for the formation of ribbon-shaped micro fibrils. The micro fibrils eventually proceed into pellicles comprised of bundles [44].

Besides, cellulose is a polysaccharide with an asymmetric chemical structure. There is a non-reducible endpoint and a reduced-function endpoint. The presence of the hydroxyl *(OH)* group is responsible for the diminished functional potential. No matter what material is used in its synthesis, cellulose retains the same chemical structure. Nonetheless, it demonstrates structural heterogeneity in mechanical, morphological, physiological, and biological domains. The availability of cellulose and the synthesis method, as well as any processing done afterward, can affect the final product's cellulose content [45]. However, in consideration of molecular structure, BC and plant cellulose except for its degree of polymerization, are identical. The polymerization degree is 2000 to 6000 for BC and 13,000 to 14,000 for plants. BC fiber looks exactly like silk fiber, which is made from the silk worm. It is a fiber with a diameter of a few nanometers [46]. For the first time in 1949, it was realized that BC micro fibrils were one hundred times smaller than plant microfibrils [47]. Micro fibrous networks are made up of nanofibers, which exist in three dimensions. This arrangement leads to the creation of hydrogel membrane which possesses high porosity and high surface area. In respect to BC features as BC is highly pure in nature so they make hydrogels with advanced properties i.e. Biocompatibility, biodegradability, high purity, high tensile strength, high crystallinity, and 3D nano fibrillar cellulosic network properties [48].

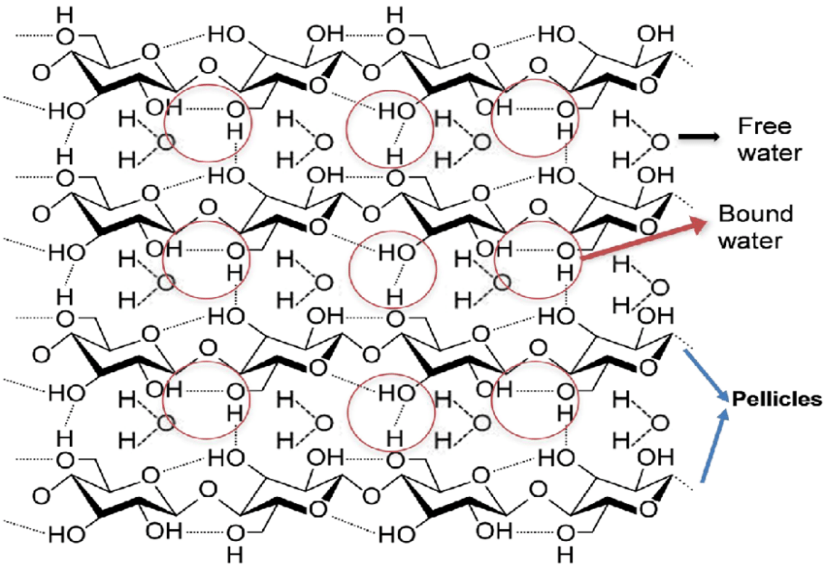

Figure 1. Chemical Structure of Bacterial Cellulose (BC) [49].

Biosynthesis

Among the biomaterials that are synthesized by Gram-negative bacteria extracellularly, some of them produce cellulose. Biosynthesis of BC is an aerobic process that, with the help of enzymatic reactions, polymerizes glucose monomers into a long chain of cellulose in microbial cells. A wide variety of regulatory proteins, enzymes, and cofactors work together to tightly control the enzymatic reactions that make up this complex process [50]. Although, BC synthesis is associated with cellular met abolism, unlike the anabolic processes involved in the synthesis of nucleic acids, lipids, and proteins, BC has no effect on these cellular processes once they have begun. It is possible to synthesize BC anaerobically using cell-free systems in addition to aerobic production by microbial cells. The assembly of cellulose nanofibrils occurs in vitro using this technique due to the presence of cellulose-synthesizing enzymes and co-factors outside of a living cell [43]. Nonetheless, the complete mechanisms of self-assembly and polymerization of glucose units remain to be discovered.

Genetic Regulation of Bacterial Cellulose

Basically, multiprotein cellulose called synthase complex (CSC) is used for the synthesis of cellulose [51]. However, the synthesizing BC and its extrusion into outside environment by microbial cells take place through a complex known as terminal complex (TCs). This synthesis process is mainly controlled by the cellulose synthase operon and is an extremely difficult procedure [10]. The cellulose synthesis operon (bcsABCD) is basically a functional unit of genomic DNA that produces Bacterial cellulose (BC). It consists of various genes. This operon encodes several genes. The proteins which accomplish a specific function for the synthesis of cellulose are encoded by these multiple genes. The gene bgIxA is present in the downstream region of these operons and any kind of mutation in this gene slows down the synthesis of BC considerably because it encodes β-glucosidase through which more than three β-1,4-glucose units are being hydrolyzed [52]. Moreover, the cellulose from UDP-glucose is synthesized by cellulose synthase. This cellulose synthase encodes four subunits (bcsA, bcsB, bcsC, and bcsD) for specific function [53]. The catalytic subunit of cellulose synthase is encoded by the first gene (bcsA) of bcsABCD operon which binds to UDPglc [54]. The regulatory subunit of cellulose synthase is encoded by the second gene (bcsB) which binds to c-di-GMP. This bcsB gene is also considered for activation of cellulose synthesis procedure and plays a significant role as a second messenger. The BcsA-B complex is activated by c-di-GMP which contains novel cellulose polymer, whose terminal glucose unit resides at a new location above BcsA's active site, where it is positioned for catalysis. Basically, polymerization reactions occur at the AxCESA protein catalytic site, which exists in the cytoplasmic membrane and is activated by c-di-GMP [55].

However, apart from the above two genes, four different genes are involved in regulating the synthesis of BC by microbial cells. These genes are axcess A, axcess B, axcess C, and axcessD and are organized in an operon. These genes encode the AxCESA, AxCESB, AxCESC, and AxCESD proteins, which are then responsible for the accomplishment of different functions [56]. Additionally, a complex is formed by protiens AxCESA and AxCESB which are encoded by the genes axcess A and axcess B. This complex is very crucial for single β-1, 4-glucan chains translocation, synthesis, and polymerization. [57]. However, the DNA sequence of these genes marks frame shift in the operon if any mutation happens in there [58]. It was also noticed that both AxCESA and AxCESB are combined in some bacterial species and coexist as a single

molecule in few bacterial species [58]. With respect to the functions of bcsC and bcsD, they are not been completely cleared. However, those proteins which are involved in pore formation or membrane channels and bcsC protein are similar which indicates that bcsC is responsible for pores formation for cellulose secretion [59]. While AxCESD is a protein (porin-like) that supports the extrusion of β-1,4-glucan chains through the outer membrane [58]. Generally, AxCESD is a protein developed as octameras and having periplasm-localized cylinder-shaped in which every octamer N-termini is placed inside the AxCESD cylinder. It forms a central pore having four pathways that are used to separately extrude β-1,4-glucan chains alongside the dimer interface in a twisted manner. AxCESD normally exists at the TCs extracellular side [60]. The functionality of this protein (AxCESD) has a relationship with the CSCs organization and the connectivity of separate microfibrils into a bigger ribbon structure [61].

Moreover, CcpAx is a small protein that is associated with AxCESD and it has a role in forming linear arrays of CSCs [62]. Other small genes include CmcAx and CcpAx that reside in upstream of the bcs operon, where endo-β-1,4-glucanase (an enzyme) is encoded by CmcAx that possesses hydrolytic activity and unexpectedly results in enhancement of BC synthesis [63]. CmcAx also causes the synthesis of twisted cellulose ribbons [64]. It is assumed that the assembling of ribbon is influenced by endo-β-1,4-glucanase. Another CcpAx gene helps in tracing the cell membrane bcs complex. Furthermore, the CcpAx also play role in transporting chains of cellulose from the locations of their polymerization inside the cell membrane and also the succeeding crystallization [65]. Besides, as the cell surface views the cellulose producing complexes as (TCs) pores and the TCs are found in all organisms which synthesize cellulose, encoded by the four genes and also a few other components i.e. CmcAx and CcpAx [62]. But some harmful effects i.e. cellulose accumulation occurs if any of the above genes are observed to be mutated or the BC synthesis is inhibited due to the inactivation of these four genes which indicates that they are very significant for biosynthesis. In contrast to that, BC synthesis is considered to be reduced by 40% due to the inactivation of acsD which suggests it plays a significant role in crystallization [58]. As a whole, the synthesis of β-1, 4-glucan chains is assisted by cellulose synthase operon. It also assists consequent extrusion through the TCs to the extracellular environment.

Synthesis by Microbes

BC is synthesized in microbial cells via a four-step process that uses UDP glucose as a precursor. First, the formation of glucose nucleotides promotes the production of monosaccharides. Second, developing cellulose chains are successively added to by repeating units of glucose. Finally, acyl groups are attached to the lone glucose molecules if they are present. The β (1- 4) glucan chains are released into the environment via the TCs terminals found on the cell membrane or cell wall [10].

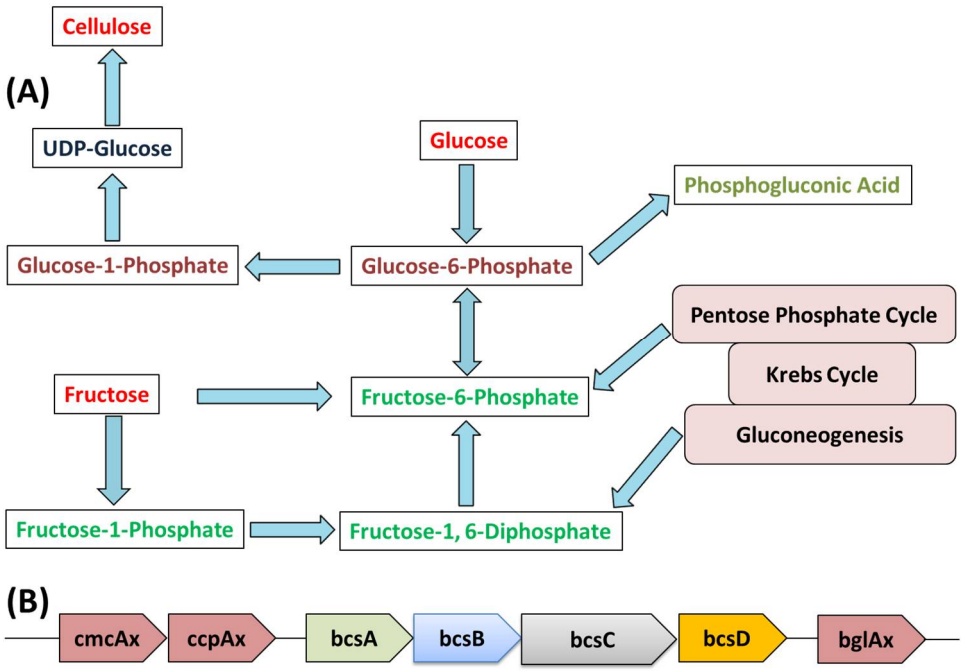

Figure 2. Biochemical Pathway for Synthesis of Cellulose and Genetic Organization of (BC).

Polymerization of individual *β (1- 4)* glucan chains leads to a ribbon-like structure, and the assembly of hundreds to thousands of such chains results in fibrils. The cytoplasm is the site of this polymerization process in its entirety. The fibrils assemble into tiny vesicles in the culture medium. Furthermore, the membrane thickens downward as more pellicles are added [44]. The same will persist until the microbial cells are silenced by the removal of all oxygen or nutrients from the growth medium or by tapping the microbial cells within the pellicles [66].

Cultivation Mode

Bacterial cellulose (BC) can be synthesized by using one of several different cultural modes, with the primary goals being to maximize productivity regardless of mode and to tailor BC's properties and applications to specific uses. BC is synthesized in a low-key manner via the static culture mode. Involving some kind of shaking or agitation of the culture, it is by far the most popular technique. Thus, the bioreactor's oxygen supply helps keep expenses down [67]. Using the static method, one can create a hydrogel form of cellulose with remarkable properties and structure. Here, in the gas-liquid phase, CO2 is generated via bacterial metabolism to create BC hydrogel according to [46]. An uninterrupted flow of oxygen into the medium facilitates price reduction, aids in increasing BC yield, and produces forms that vary in appearance based on the rate of rotation [68]. This mode of synthesis is thought to be useful in fields like regenerative medicine and tissue engineering, but its low production yield and time consumption may prevent it from being used in industrial settings. Fed-batch cultivation is an alternative method that may increase yield sufficiently [69].

Also, a stirred-tank bioreactor can be used to produce fibrous BC suspensions with a low elastic modulus, low crystallinity, and low polymerization degree, while an air-lift bioreactor is another type of reactor that permits a continuous transfer of oxygen within the culture medium and thus a sufficient supply of oxygen. Compared to stirred-tank reactors, this is thought to be more efficient with energy. Since unmodified BC is deficient in many desirable qualities including antioxidant, antimicrobial, electro conductive, and magnetic, a variety of industrialized methods have been developed to introduce materials into its matrix and transmit additional features for a wide range of applications [70].

Applications in Biomedicine

Bacterial cellulose (BC) is considered a multipurpose polymer for its use in several biomedical applications as BC is highly pure, nontoxic, and also biocompatible The production of BC-based composite scaffolds compounded with additional constituents like polymers and nanoparticles has led to the development of several effective biomedical products [71]. The molecules of plant cellulose and bacterial cellulose are indeed identical, but BC is more appealing to the scientific community because of its many desirable qualities. These include biocompatibility, high crystallinity (70%-80%), thermostability, mechanical stability, high purity, high specific surface area, high porosity, outstanding permeability, and high-water content (up to 99%). [72].

BC's initial application was its extensive use in the making of coconut gel, commonly called nata de coco. A fermentation technique, primarily commercialized in 1973 in Philippines, through which luminous jelly-like food was created. This food was created from the fermentation of coconut water by bacteria [46]. However, as an alternative to plant cellulose, BC also possesses some precise biomedical applications [73] i.e. food processing, packing, cosmetics, and also some other applications.

Wound dressing, clothing base, and paper binding agents are just a few of the more recent medical and commercial uses for BC. Wound tissue engineering, targeting cancer, drug delivery systems, biosensors, skin, etc are just a few of BC's many medical uses. Artificial blood vessels, surgical mesh, dental implants, wound-dressing materials, bone stuffing, meniscus, heart valve, casing in nerve surgery, artificial cartilage, arterial stent coating, etc. are all being developed using bacterial cellulose [12]. However, BC has other potential applications in the food industry. Cellulose nano crystals and natural nano fibers have recently been demonstrated to have the potential for use in the formation of nano composite films and coatings. Because of this, it was suggested that CNCs be made from a variety of materials via chemical, biological, or chemical treatment methods [74]. Some of BC's most significant medical applications are shown in Figure 3.

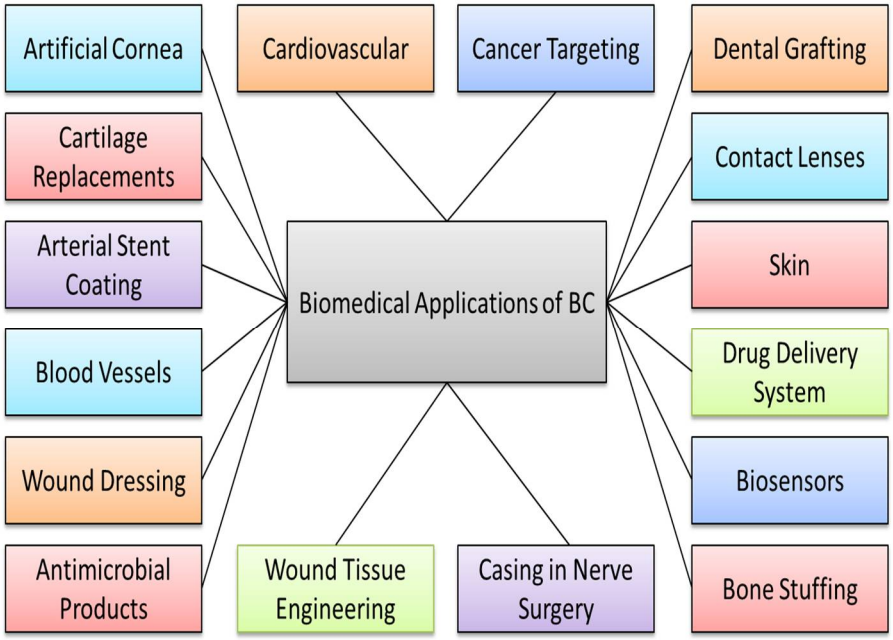

Figure 3. Biomedical Applications of Bacterial Cellulose

Using a Particular Characteristic

BC is used in many applications in food, the environment, and optoelectronics energy due to its unique structural, mechanical, rheological, thermal, physiochemical, and biological properties, which encounter for its sustainability or suitability in numerous applications [10]. BC, which is made in a cell-free system and from microbial cells, needs to be characterized in different ways with regard to its various properties, as we have already discussed.

Scanning Electron Microscopy

In an investigation, with an intensive beam of electrons, the scanning electron microscope (SEM) scans the surface of a material. The electrons interrelate with the atoms in the material, thus producing an image by developing a signal holding information about the composition, cross-sections, and surface topography [75]. The information about the compactness, density, thickness, and relaxation of micro fibrils is provided by SEM. It also provides information about length as well as their degree of branching [76]. Regardless of all the above, information analysis through SEM is restricted to inorganic and solid samples that bear a temperate pressure and are minor in size to fit easily inside the vacuum chamber. Therefore, as BC is extremely elastic and also vacuum resistant, it can be easily characterized through SEM.

Transmission Electron Microscopy

Basically, TEM provides evidence related to the elemental composition of membrane materials. In an investigation based on TEM, a beam of electrons refocused, transformed through the material, and is magnified by an electromagnetic system. Then the beam of electrons is focused onto a phosphor screen for altering the electron image into a visible image [77]. TEM also provides evidence regarding the crystalline structure and structural morphology [78]. Structural information about BC Nano fibers is obtained through TEM analysis. Typically, TEM analysis is not accepted in terms of its use for living organisms because the preparation of the material needs fixing with some cryogenic techniques or chemicals which are not suitable for them and ultimately damages the cells [10].

Methods Using the Fourier Transform Infrared Spectroscopy

The Fourier transform infrared (FT-IR) spectrometer is a powerful instrument for studying the emission and absorption of infrared light at specific frequency bands associated with chemical bonds. FT-IR technique perceives the distinctive vibrations of chemical bonds and is mainly based on the infrared radiation absorbed by the analyzed sample [79. The absorbed spectrum can be used to help characterize the chemical makeup of even the most complex of samples. [80]. There is no chemical difference between the celluloses represented by the FTIR spectra generated in microbial cells and cell-free systems, whether they are grown in different media or the same media like plant cellulose [10].

Diffraction of X-rays

The x-ray diffraction method is used to determine the atomic and molecular structures of crystals and provides additional confirmation of the samples structure and crystallinity. However, it does not provide information about its chemical nature [81]. X-rays are generated by a cathode ray tube and are based on their constructive interference. Firstly, x-rays for the production of monochromatic radiation are filtered, paralleled to direct or concentrate them, and then focused on the sample causing the rays to spread in several directions, and it depends on the solid internal structure [10]. In the case of BC, from sample to sample ratio of crystallinity to amorphous areas fluctuates and depends on several conditions, i.e. the selected microorganism, the composition of media, and also on conditions for its processing [67].

The Results of a Tensile Test

The mechanical behavior of isotopic materials is typically determined through tensile test analysis, such as uniaxial testing, which defines material characteristics like deformation, relaxation, and elongation. By applying a force or load, a tensile test analyzes how far a material can be stretched before breaking. In most cases, the material broke in three stages: necking, cavity formation, and introducing the interior cross-section of the material with micro voids [82]. A mechanical test is performed on materials in the case of BC, and due to its randomness, it is difficult to twitch or pressurize individual cellulose fibers [83].

Nanoparticles

Nanotechnology is extended massively and gaining more attention with time, producing material at a nano scale. Nanotechnology relies heavily on nanoparticles, which are the particles between 1 and 100 nanometers in size. Nanotechnology was first introduced by *Nobel laureate Richard P. Feynman* in his 1959 book (There's Plenty of Room at the Bottom) [84]. Metal, metal oxides or organic matter, and carbon make up these nanoparticles. Nanoparticles have unique chemical, physical, and biological properties compared to bulk particles because of their large surface area to volume, superior mechanical strength, and increased stability or reactivity in chemical processes. Nanoparticles are used in a wide variety of fields because of their exceptional characteristics. Nanoparticles are distinguished from one another not only by their composition, but also by the fact that they range in size, shape, and number of dimensions [85]. Realizing that their size can affect a substances physiochemical property, such as its optical properties, increases the importance of these substances to the scientific community. Several revolutionary advances have been made in the field of nanotechnology. Nanoparticles come in a wide range of sizes, shapes, and structures, including spherical, cylindrical, hollow-spiral, tubular, conical, flat, irregular, and spiral forms, among many others [86].

In Figure 4, we see that nanoparticles can have any of the four possible dimensionalities: 0D, 1D, 2D, or 3D. In the case of 0D, i.e. nano dots, the dimensions (width, length, and height) are all equal. Graphene, a 1D material, owns only the thickness parameter, while 2D carbon nanotubes, own both the length and width parameters [87]. Gold nanoparticles are three-dimensional objects, meaning they have depth, width, and length. The surface is not uniform and displays a range of irregularities. NPS have a complex structure with three layers, so they cannot be reduced to the status of simple molecules. One of them is the surface layer that might be functionalized with a diverse variety of polymers, small molecules, surfactants, and metal ions. The second layer is the core, which is basically the central portion of NP. Finally, the shell layer shows fluctuation from the core chemically in all features [88]. These materials got a huge attention of scientists due to their unique characteristics in multidisciplinary fields.

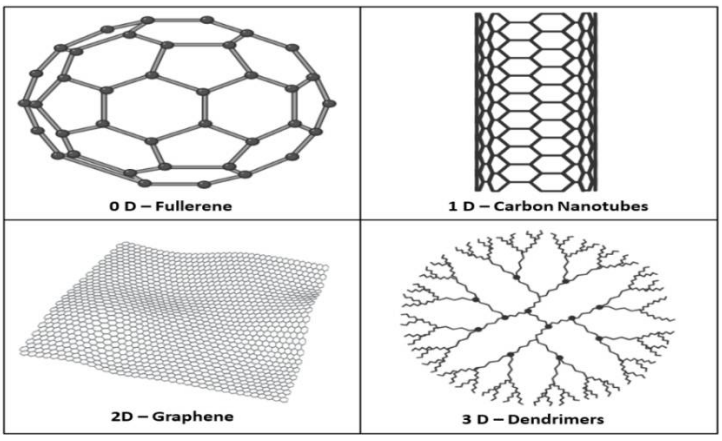

Figure 4. Schematic Illustration of Nanoparticles based on Dimensions (0D, 1D, 2D, 3D).

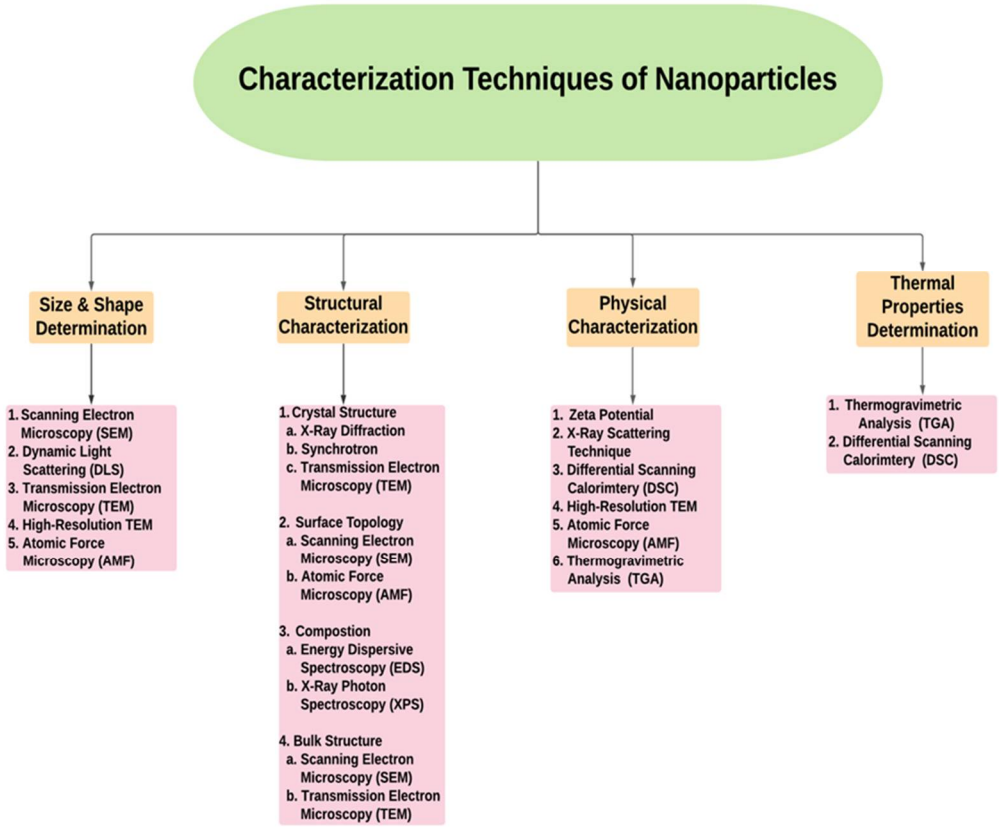

Figure 5. Characterization Techniques for Nanoparticles

Many different production methods are developed or modified to achieve targeted nanoparticles with improved chemical, physical, and mechanical properties, all to lower production costs. Enhanced characterization techniques (Figure no.5) and application of nanoparticles are also conceivable because of massive progress in the instrumentation [85] Additionally, mesoporosity bestows additional properties upon NPs. Drug delivery [89], gas sensing [90], carbon dioxide capture, and other uses are just a few examples of the many possible applications for the NPs.

Types of Nanoparticles

Based on the classification, nanoparticles can be categorized into organic, inorganic, and carbon carbon-based, as discussed below.

Nano-sized organic particles

The most common organic nanoparticles or polymers are liposomes, micelles, ferritin, and dendrimers [91]. Some of these nanoparticles, like micelles, also called nano-capsules, have a hollow core that makes them sensitive to electromagnetic and thermal radiation (like light and heat), while others, like the bulk of nanoparticles, are inert [92]. These distinguishing features make them a promising new drug delivery option. Drugs normal features, like surface morphology, size, etc., are not the only things that determine their delivery capacity and stability, these factors also determine the drugs efficacy in the field [93]. Organic nanoparticles are widely used for drug delivery due to their efficiency and the fact that they can be injected into a wide variety of body sites (targeted drug delivery).

Nanoparticles of inorganic substances

In general, metal and metal oxide nanoparticles belong to the inorganic category. In addition, carbon is not a component of these nanoparticles.

Nanoparticles made of metals

Metals are synthesized into nanoparticles using either destructive or constructive methods. While nanoparticles of virtually any metal can be synthesized, the most common ones are those of aluminum, gold, cadmium, copper, iron, lead, cobalt, zinc, and silver [93]. These nanoparticles are distinguished from others in several ways, such as their size (typically between 10 and 100 nm), surface area to volume ratio, amorphous and crystalline structure, spherical and cylindrical shapes, sensitivity to the surrounding

environment, etc. This class of nanoparticles can be used in a wide variety of chemistry and medicine applications [94].

Sub-microscopic Particles of Silver

Given that silver nanoparticles (AgNPs) have unique electronic, antibacterial, and optical characteristics, they are widely used in bio-sensing, electronics, photonics, and antimicrobial applications [95]. The unique broad-spectrum antimicrobial activity of these (AgNPs) nanoparticles is of great importance in the design of these nanoparticles, which includes food storage containers, catheters, bandages, and antiseptic sprays. Due to their potential therapeutic uses, such as their effectiveness as anticancer agents, silver nanoparticles have recently attracted a lot of attention [96]. Silver has unique material features, is cost-effective, and available in an abundance of natural resources, but on the other hand, the usage of silver-based nanoparticles has some limitations because of their instability, for instance, the oxidation in an oxygen-containing fluid [97]. As a result, silver nanoparticles have lower potential as compared to relatively stable gold nanoparticles. In addition, silver nanoparticles have antimicrobial applications that possess recognized antimicrobial properties of Ag+ ions [98].

Nanoparticles composed of metal oxides

Nanoparticles made from metal oxides differ in important ways from their metallic analogs. The synthesis of metal oxide-based nanoparticles modifies the properties of metal nanoparticles; for instance, Iron nanoparticles are oxidized to Iron Oxide in the presence of Oxygen at room temperature, increasing its reactivity. Metal oxide nanoparticle synthesis is motivated by improved efficiency and increased reactivity [99]. Silicon dioxide, cerium oxide, iron oxide, aluminum oxide, titanium oxide, magnetite, and zinc oxide are among the metal oxides that are most frequently synthesized [100].

Nanoparticles of carbon

Nanoparticles based on carbon are composed entirely of carbon [99]. Nanoparticles can be manipulated in many ways due to their malleability in shape, size, and structure. Different types of nanoparticles, such as carbon nano-fibers, carbon nano-tubes (CNT), graphene, fullerenes, and nano-sized activated carbon, exist. Unfortunately, all forms of carbon nanomaterials aggregate and tend to exhibit some undesirable properties. Since carbon nanoparticles do not tend to aggregate and are only moderately soluble or soluble in polar solvents, researching their activity is a challenging endeavor [101].

Biosynthesis

Synthesis strategies for nanoparticles can be broken down into two distinct categories: bottom-up and top-down. The top-down method involves reducing or dropping bulk material into nanoscale particles, while the bottom-up or constructive method involves building up from atoms to clusters and then to nanoparticles. The bottom-up approach is also called the constructive method, while the top-down approach is called the destructive method [93]. Figure 6 below depicts the bottom-up and top-down approaches;

Composites

Synthesis of new materials with the potential to improve the eco-friendliness of products has become a major focus of modern research. Natural raw fibers and polymer matrices have been used in the synthesis of composites because of the demand for eco-friendly or green materials worldwide. In recent years, polymer-based materials with natural-organic fillers, such as those derived from biodegradability or renewable sources, have gained a lot of attention [102]. A composite is typically made from multiple components. Composite material, such as resin, is formed through the combination of two or more constituents, typically a reinforcing agent, such as a fiber, and a matrix binder, such as plastic. To achieve the desired properties and characteristics, the constituent parts of a composite may either completely dissolve into one another or work together in some other way. Natural fiber composites, on the other hand, can be used as a replacement for synthetic materials that are harmful to the environment, thereby reducing pollution and other related issues. It has also better mechanical properties.Additionally, it requires low amounts of energy to manufacture [103]. Good mechanical properties, higher fracture resistance, enhanced electrical resistance, good thermal and acoustic insulating properties, and low thermal expansion and contraction are just a few of the unique characteristics of natural fiber composites [104].

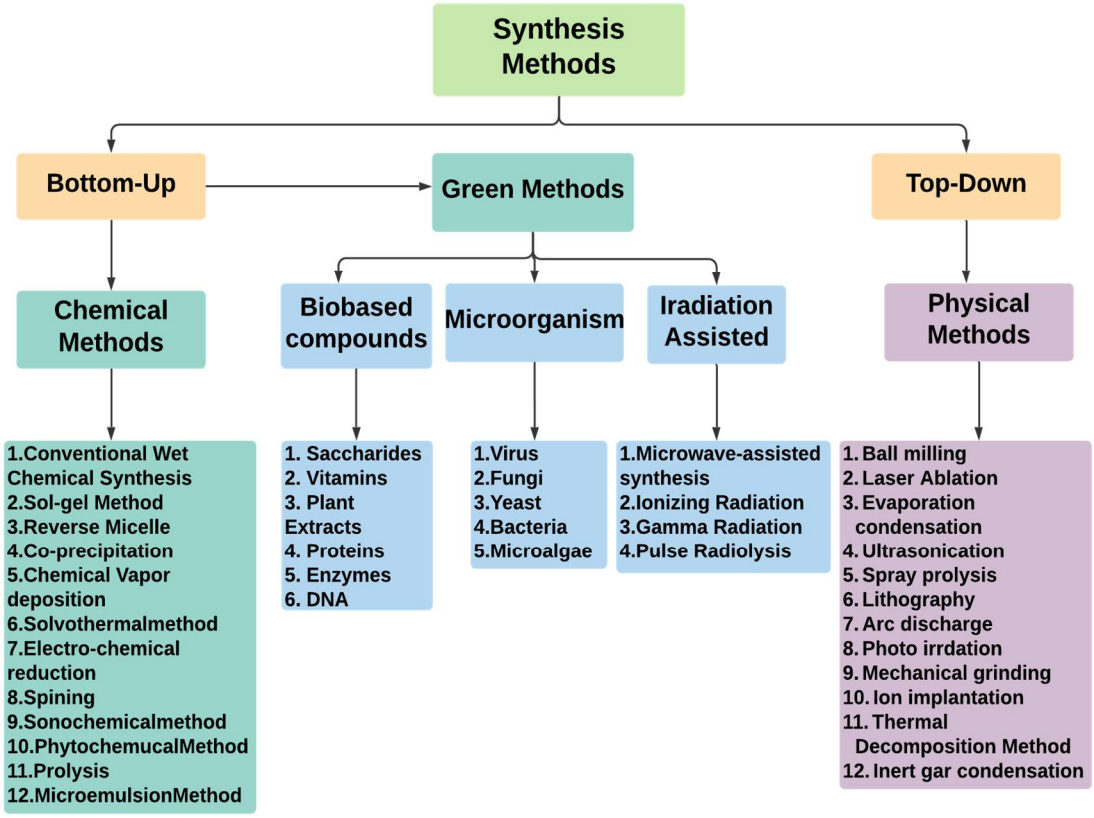

Figure 6. Bottom-up and Top-down Synthesis Methods of Nanoparticles

Developing Hydrogel Composites from BC and Nanoparticles

Bio-nanocomposites are solid mixtures of inorganic and biopolymers that find widespread application. They have antimicrobial activity, biodegradability, and biocompatibility, and are typically in the nanometer range (1-100 nm) [105]. Biohybrids, green composites, nanocomposites, biocomposites, and bionanocomposites are all terms that are often used interchangeably with bionanocomposites. Studies based on bionanocomposites are mostly before 2004 [106]. Wagner began manufacturing the reinforcement material in 1941. Composites are created when he adds silica nanoparticles (10-100) to a natural fiber as a reinforcing material [107]. Bio-nanocomposites can be broadly categorized according to their shape, origin, matrix type, and reinforcement size. Bio-nanocomposites can be further subdivided into Particulate bio-nanocomposites, elongated particle bio-nanocomposites, and layered particle reinforced bio-nanocomposites, depending on the shape of the reinforcement particles [108].

There are two main things to keep in mind when working with composites that are based on bacterial cellulose (BC), even though BC is a great biopolymer. Because of its well-ordered web-shaped fibrous network, BC is widely regarded as having ideal structural features for the synthesis of BC-based composites with multiple materials [109]. These BC fibers serve as a matrix because they can enclose nanoparticles within their structures. The presence of hydrogen bonds in cellulose chains allows for interaction with other polymers, resulting in robust composites [73]. Not only is BC hypoallergenic, but it also lacks cytotoxicity [110]. Second, pure BC is lacking in certain features that are essential for certain applications across various fields. As a result, fewer BC applications will be possible. On the other hand, BC properties, in the sense of physical and chemical properties, can be enhanced through composite preparation with several materials like collagen, gelatin, chitosan, sodium alginate, and polyethylene glycol. Collagen (COL), chitosan (CH), and gelatin have all been successfully combined with BC to improve their biological properties, even though many different polymers have been used in the synthesis of composite [66]. It is the combination of BC, alginate, and chitosan that inhibits the growth of pathogenic microorganisms like *Candida albicans* and *E. coli*; BC itself has no antibacterial properties (111). Despite this, BC composites containing polyaniline (PAni) and graphene oxide (GO) enhanced BC's conductive properties [112].

To date, scientists are attempting to enhance the BC's mechanical and magnetic properties, biocompatibility, conductivity, and bactericidal activities through the synthesis of composites with a wide variety of materials [113]. Although BC is a biopolymer with promising biomedical applications and

demonstrates biocompatibility, its use is limited by a lack of certain properties. So, to define these bounds, BC composites synthesis is critically important [66]. Therefore, nanoparticles have been progressively integrated into biomaterials due to their specific nano-scale properties and significance as a component of medical devices. Increased chemical reactivity, meaningful changes to Physico-chemical properties due to a large ratio of the surface atom, rapid dissolution in some cases, altered and electronic properties, etc. are all hallmarks of nano-scale materials compared to bulk size, and these properties can be very useful in biomedical applications [114]. If they are meant to be released inside the body, their smaller size makes that possible more quickly. In contrast, embellishing the surface of any nanoparticle will change its surface properties. This modification will lead to a changed biological behavior finally. It will also have a clear effect on their materials, and because of this reason, in the case of treating filthy wounds, BC composites must be considered. Gold (Au), silver (Ag), platinum (Pt), palladium (Pd), titanium oxide (TiO2), and iron oxide (FeO) are just some of the nanoparticles (NPs) used in the production of BC composites [115].

Synthesis of BC Composites

The distinct features of BC that are influenced by the fabrication process motivate or make the way towards the synthesis of BC composites During its creation, the micro fibrils are arranged in a web-like structure. Because of BC's porous nature and hydrophilic characteristics, material saturation and adhesion are facilitated rather than complicated, allowing them to incorporate a wide variety of materials into culture media [116]. Therefore, BC is considered a biopolymer that can be subjected to a wide range of polymer synthesis methods. BC's structural properties allow it to function as both a reinforcement agent and a matrix in the synthesis of composite materials, making it a versatile material. The biomedical applications, biological activities, conductivity, magnetic properties, and mechanical properties, however, have all been enhanced by the use of different BC composites [117]. Based on the properties of the composite materials and their potential uses, synthetic approaches have been developed for BC composite synthesis [118]. Both the in situ (adding reinforcement materials to BC synthetic media) and ex-situ (penetration of that kind of materials into BC microfibrils) methods can be used to create BC composites (Figure 7). [119]. However, solution mixing and polymer blending are two synthetic methods that seems much less use. Materials ranging from organic polymers to inorganic nanoparticles can also be considered for use in the synthesis of composites [66].

Synthesis of Carbon Fiber Reinforced Plastics in Situ

The *in-situ* process causes insolubility of several constituents in culture media. This is because of the antibacterial characteristics of fortifying agents that have the capability to destroy the BC developing cells [120]. At the beginning of the procedure reinforcing agents are introduced into the culture media in this approach and then BC micro fibrils become dense and a web-shaped structure is formed with the progress of time. During the synthesis of BC, in this unique web-shaped structure, different materials can be confined or trapped [76]. Since the particles in BC synthetic media just remain suspended for some time, the synthesis of BC composites via static culture is a challenging process. On the media surface, the BC sheet developed at the air-media interface. Particles cannot be trapped on the surface of BC fibers if they sink through the medium or precipitate out of the solution. But agitation culture provides a better situation for entrapping the materials that are added into BC fibrils, as the material makes a constant effort of moving in the media, which prevents the particles from settling. Particle entrapment is maintained due to persistent BC growth and spread [121].

That is why in-situ BC composite synthesis is so common; it requires a wide range of flexible operating procedures and agitation tools [66]. Some significant bioactive (antibacterial) agents, such as antibiotics, cannot be added directly to the media, which limits the number of BC composites that can be produced using the in-situ technique (Ag, ZnO, TiO2). They have some lethal effects on microorganisms [66]. Furthermore, sheets or gels of BC composite made via agitation culture cannot be used in biomedical applications. While sheets and gels can be formed in static culture but they cannot capture reinforcement materials for the synthesis of composite.

Creating BC Composites Outside of a Mold (Ex-Situ)

To resolve the issue that is associated with the synthesis of BC composite through *in-situ* technique. Nanoparticles and liquid are integrated into the structural matrix of the BC prepared [73]. The permeable network of BC provides space for fertilizing other substances inside the network. The materials either engaged in BC composite or through forming chemical bonds with the polymeric chains of cellulose, depending on the chemical structure [122]. The primary advantage of the ex-situ method is that it preserves the original BC structure [66]. When preparing composites for use in industries and healthcare, static cultivation of BC sheets is a key step [123]. There have been a lot of BC-based composites that have used this approach. Metal-based, metal-oxide-based, inorganic metal, and polymer-based BC composites [73]. The properties of BC are modified in various ways depending on the reinforcement material used. BC

with CNTs, chitosan, gold nanoparticles, gelatin, polyethylene glycol, silver, etc., is all part of some of the BC composites made with ex-situ synthesis methods [124].
But the main drawback of this method is that some particles of a certain size infuse into the BC fibers. In addition, the hydrophobic materials did not easily combine with BC in the composite preparation process [125]. Ex-situ composite synthesis presents additional challenges related to the nature and size of reinforcement material, as only submicron to nano-sized materials can be infused or impregnated into the BC matrix. This is because hydrophobic materials are incapable of combining with BC, and larger particles could not enter the BC pores ([66]. Because the BC fibril structure is not regularly constant, the penetrating materials may also not be uniformly distributed within the BC matrix.

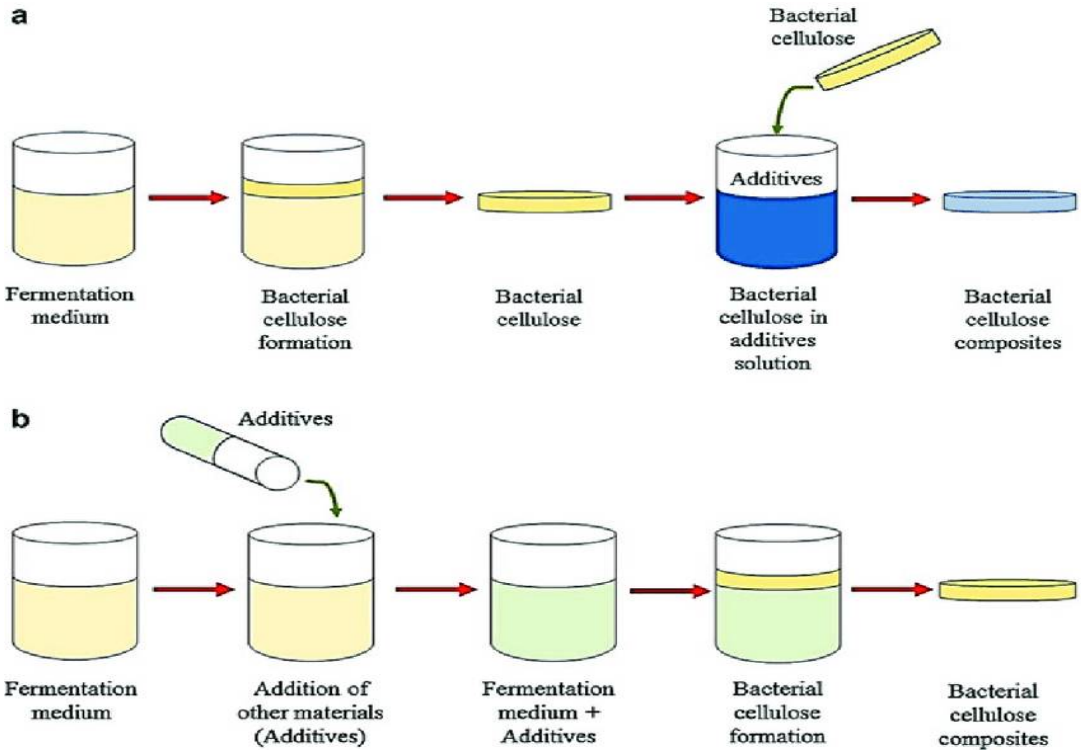

Figure 7. In-situ and Ex-situ Methods for Bacterial Cellulose Composites Synthesis [126].

Clinical Relevance

As stated before, research areas are making continuous efforts in the usage of bacterial cellulose (BC) in a vast variety of biomedical devices and materials i.e. medical implants, scaffolds for tissue engineering, and wound dressings. Accordingly, researchers are focusing intensively on making composites for improving BC properties. Because of its wide range of potential biomedical uses, many BC-based composites have been developed.
Cellulose-based superabsorbent hydrogels have many uses in biomedicine, including as cell bioreactors, drug delivery vehicles, tissue engineering scaffolds, and micron-patterned media for neural cell cultures [127]. Nanocellulose's emerging nano features and properties make it useful in a variety of biomedical research fields, including cell and gene therapy, as well as controlled and diagnostic delivery [128]. Several different types of nanoparticles, including silica, carbon-based, metal, and polymeric nanoparticles, can be incorporated into BC composites for use in a variety of analyses [129]. Microbes are eliminated due to the interaction between nanoparticles and the cell membrane, which compromises the viability of the cell and its ability to respire. One of the most promising uses for BC is as a temporary skin substitute in the medical field for the treatment of burns, ulcers, and wounds [130].
In middle-class counties, around 90% of deaths take place due to burns and about 300,000 people die all around the world each year [131]. That is why Burn injury is considered as the furthermost hurting trauma. Mostly, first-degree burns mostly rebuild quickly and without any difficulty or complications. However, full-thickness and partial burns are very complex and are a crucial challenge in clinical areas to deal with it because the changed anatomic structure and ultimately a function of skin leads to loss of fluids heavily in case of burns. This injury suffers from infectious complications for example, cellulitis, invasive bacterial

infections, and impetigo [132]. For all the reasons, BC possesses various intrinsic features that reassure it use in wound healing or dressings. Though, BC itself does not show antibacterial property which is needed in wound treating application [130]. Therefore, different approaches have been used to address the problem that mainly consists of biocidal polymers-BC composites, antimicrobial agents functionalized-BC, NPs impregnated BC, and metal or metal oxide. In this regard, the synthesis of antimicrobial BC membranes is in progress devotedly, especially BC having silver particles [133].

Nanomaterials are widely used as novel antibacterial agents in the fight against infectious pathogens. Biocompatibility and antimicrobial activity in vitro have been demonstrated for BC composites containing nanoparticles. Polymers serve as the backbone of most modern bio composites, which also typically include particles or fibers that modify the systems thermomechanical or biological properties [110]. When the cut is minor, the skin usually mends on its own. When there is full thickness, when the area of missing skin is more than 4 centimeters in diameter, complications arise [134]. Thus, a graft is crucial for wound healing. The presence of infection-causing microbes in a wound can also delay the healing process and increase patient suffering [135]. BC is commercialized as a bandage or dressing for burn wounds because it is widely regarded as a skin substitute in the biomedical industry [136]. For the treatment of burn wounds, for instance, [137] created a BC-based nanocomposite material with polydopamine (PD)-coated silver nanoparticles (BC-PDAg). Using in vitro studies, they demonstrated that the BC-PDAg nanocomposite is biocompatible and promotes cell growth.

Additionally, silver nanoparticles are known to exhibit strong cytotoxicity to a wide variety of microorganisms because they are effective at creating a bactericidal (antibacterial) and bacteriostatic (growth-inhibiting) impression based on the oligodynamic effect [138]. They are infused with BC to achieve the antimicrobial activity, as silver is bactericidal. A composite with antibacterial properties was an aim of *Volova et al*. This is why they added a silver nanoparticle to the BC for added strength. When applied as a bandage, this composite helps clean up and heal infected sores and wounds [110]. Silver nanoparticles have enhanced antimicrobial properties because of their high specific surface area and a high fraction of surface atoms, two of their most prominent features. The antifungal properties of BC-Ag composites, on the other hand, are quite promising [139]. Moreover, a hydrogel based on BC and collagen (BC/COL) has been proposed as a wound dressing. When compared to collagenase, BC/COL hydrogel was found to be significantly more effective at healing the wound. As a result, it is being evaluated as a potential dressing for wounds in skin regeneration [140]. Similarly, BC and vaccarin (BC-Vac) membranes were developed successfully on a large scale for use as wound dressing materials because BC-Vac used in wound healing treatments demonstrates that wound is epithelialized and regenerated more rapidly than that treated only with BC and also shows no cytotoxicity [141]. Furthermore, one of the recent studies demonstrates the use of a never-dried BC membrane in the treatment of a patient with severe second-degree burns [142]. Membracell®, Xcell®, Bionext® [143], and Biofill® [144] are just some examples of BC-based commercial medical products. As a result, the product has been modified in an antimicrobial fashion to reduce the risk of infection in wounds and as a cutting-edge means of addressing it [145]. To that end, the hydrogel was proposed as a prototypical wound dressing because of its ability to retain an adequate supply of moisture while still allowing the injured area to breathe [146].

Regarding BC hydrogel, after the extraction from the culture medium, it sustains its extraordinary flexibility. Because of its adaptability, BC can be used in any kind of wound, no matter where on the patient's body the injury occurred [145]. High compatibility with the wounds exterior has led scientists to develop BC-based composites as a wound dressing material [147]. It has been found that BC-composites used as wound dressings provide a significantly higher level of patient comfort than conventional wound dressings [145]. Further, BC-based composites have been validated in the literature as having a dual role as wound dressings and drug carriers, both of which improve full-thickness wound healing [71]. Inorganic nanoparticles have attracted interest for their potential applications because of their unique electronic, magnetic, optical, and antibacterial properties [148]. So, oxides of titanium (TiO2), magnesium (MgO), and copper (CuO) are used for thorough research into biosensors, biocides, and a few other cutting-edge applications. Despite the extensive use of metal oxides (ZnO, Au, CaO) in a variety of remarkable applications (biosensing, cell imaging, cancer therapy, gene or drug delivery, etc.), bio-inorganic nanocomposites continue to draw more and further attention [149]. ZnO is also unique in that it is an inorganic metal oxide. For this reason, it has recently attracted a lot of attention. It is more physically and chemically stable than its competitors. Because of its many useful properties including photo-catalytic activities, semi-conducting properties, infrared and intense ultraviolet (UV) adsorption, efficiency, nontoxicity toward animals and humans, and an antibacterial function, ZnO is often referred to as a "multifunctional material" [150]. Recent studies on ZnO nanoparticles have revealed promising applications in cancer treatment [31]. As ZnO nanoparticles have antibacterial properties, BC composites

containing these nanoparticles may be used as remarkable materials in wound healing procedures due to their outstanding features. As a result, it facilitates recovery more rapidly. Most importantly, unlike other nanoparticles, BC-ZnO nanocomposites pose no health safety concerns. In addition, it has many potential applications in the manufacturing sector [151]. On the other hand, BC-Au nanocomposite exhibits unique concern in certain biomedical applications, particularly enzyme immobilization procedures [33]. It can also be used in biosensors.

Furthermore,the in situ radical polymerization strategy used to prepare poly (2-hydroxyethyl methacrylate) (PHEMA) based BC composites have been shown to have no toxic effects on human adipose-derived mesenchymal stem cells (ADSCs) [152]. BC-polyethylene glycol composites, as opposed to pure BC, have shown significantly higher cell proliferation and biocompatible properties. Furthermore, montmorillonite (MMT) and altered MMTs with nanocomposite BC have demonstrated enhanced biomedical potentials, specifically potent bactericidal activity [73]. *Millon* and *Wan*, two other researchers, set out to create BC-polyvinyl alcohol composites with mechanical properties similar to those of natural heart leaflets [153].

In addition to these fields, biomedical applications involving Fe3O4 nanoparticles, such as gene and drug delivery, have garnered considerable attention. The electrical stimulation of cells and tissues is one of the most important biomedical applications of nanocellulose/CNT composites, which can be used to improve cellular function and tissue regeneration through tissue engineering [154]. For instance, bio-patches that are stretchable, electrically conductive, and flexible can be used to stop arrhythmias and restore conduction in damaged cardiac regions. The incorporation of chitosan into the composite improved the cell adhesion, biocompatibility, and proliferation properties of BC, making it useful as a wound dressing material and a scaffold in tissue engineering. It can store water for long periods and release that water very gradually, according to research by Ul-Islam et al., (2015). As Kim et al., (2011), proposed in their study, BC/gelatin composites exhibit proliferation and fibroblast adhesion under optimal growth conditions (Ul-Islam et al., 2015). Also, they exhibit better biocompatibility than pure BC, and the developed scaffolds were bioactive, suggesting they may be useful as wound dressing [33].

As biomedical advances have allowed for more efficient transport of therapeutic agents, new methods of treatment and protocols are constantly being developed. BC has some exclusive characteristics that make it important for applications in drug delivery systems i.e. large surface area and tremendous drug loading capacity [155]. Composites have an equally important role in the delivery of drugs. Peres et al, developed a photosensitizer, color aluminum phthalocyanine (ClAlPc) based BC membrane, and used it to create a drug delivery system for photodynamic therapy (PDT) of skin cancer. To accomplish retention and skin permeation in their in-vitro study, pig ears are used as skin models. There was evidence that ClAlPc's properties were suitable for use in a topical formulation. A lack of cytotoxicity was observed in BC-ClAlPc membranes in vitro assays [156]. Additionally, the main benefit of BC in the context of drug delivery is its biocompatibility. This led to the development of a polyvinyl alcohol (PVA)-based BC composite with excellent biocompatibility. It is crucial for drug delivery that this composite is reported to be biocompatible [152]. Likewise, Ullah et al., (2017) concluded that non-modified BC was not an appropriate vehicle for the delivery of control drugs. Since it still has a porous structure, it can be easily permeated by gases, solvents, and other small molecules, but it does not tolerate their passage. In order to prepare and evaluate BC matrices with the modified surfaces for drug delivery applications, [157] also prepared and tested a control group. Also, the FDA has certified medical implants and devices made in British Columbia (FDA). These properties make it possible to apply BC scaffolds in transdermal settings, in drug delivery, and in tissue engineering [143]. Since transdermal and oral delivery are typically used for medications, it is possible to combine drug delivery and tissue engineering in the case of various drugs. It highlights the promise of BC for use in drug delivery. Furthermore, when there is a successful modification of the BC surface, there will be more time for retention for effective drug delivery. It has thus been suggested that BC can be modified to control and prolong drug delivery [158]. It has also been reported that BC-based composites improve their anti-tumor effects by boosting cell apoptosis and reducing cancer cell growth [147]. This is why these composites play a role in transdermal drug delivery. Therefore, BC-based composites exhibiting a 3-D structure have shown the ability to mimic the tumor cells' 3D growth [33].

In consideration to hydrogels for drug delivery, hydrogels consist of hydrophilic polymer chains which are water-swollen polymeric materials with the display of 3-D network structure [159]. Their hydrodynamic properties are the same as biological tissues therefore they can be used efficiently in drug delivery systems by loading drugs in their network. They are appropriate for topical application through skin hydration to assist the permeation of drugs in the skin [160]. The BC-chitin-curcumin composite film has upgraded the physicochemical properties compared to BC. It is reported that drug delivery applications, they could be a promising active film [161]. Similarly, BC–graphene oxide (GO) composite is found in applications in

pharmaceutical, medicine, and industries, though BC-GO nanocomposite is considered a novel drug nano-carrier. It has the capability for delivery of drugs and based on mechanical properties, it can be used as promising material applications like replacement of cardiovascular soft tissue [162]. In addition, the biocompatibility, drug release, and bactericidal activity of BC composite membranes containing tetracycline hydrochloride (TCH) were evaluated [163]. The release of drugs is an appealing aspect of BC, in addition to drug loading. Within 24 hours, 90% of the drug release progressively loaded in the BC. This drug-loaded BC quickly demonstrates bactericidal activity, providing proof of the BC's potential release and nontoxic biocompatible behavior during drug loading. The use of BC-based composites for drug delivery has increased dramatically in recent years due to their unique properties [164]. BC-based composites can be used as carriers for a wide range of drugs, and the drugs themselves can come in a number of different forms, including microspheres, tablets, pellicle (film), and hydrogel (gel) [165]. Even though BC and other inorganic and organic materials make up these composites (such as graphene, chitin, chitosan, CMC, etc.).

RECENT PROGRESS

Recent interest in the design of natural materials has been driven by sustainability and has focused on a wide range of potential uses. New processing, manufacturing, and functionalizing methods for biomaterials have made structural specificity possible thanks to advancements in materials science [166]. Bacterial cellulose (BC) has been explored by researchers for furthermore application in the biomedical field as it is considered a wonderful material. Hence BC has gained much more accomplishment in the biological medicine area and some other fields. Apart from pure BC, its structure provides a much better atmosphere for developing composites. As composites are synthesized to deliver some other qualities in original materials which are significant for specific applications but they did not have before on its own. Thus this quality further expands the BC applications.

Recently, [167] developed a chitosan-alginate-BC composite by treating chitosan with hydrogen peroxide. This composite material perfectly matched all the mechanical requirements for use as a wound dressing. There is also evidence that it can rehydrate when dehydrated. These characteristics are critical for a wound dressing because they aid in soaking up excess exudates. Nonetheless, the controlled release of drugs through the use of these chitosan/alginate/bacterial cellulose composites was later validated [167]. In addition, the translucence of the silver nanoparticles-based composite allows for routine wound monitoring without removing the dressing [168]. It has also been claimed that gold nanoparticle-based bacterial cellulose composites have a more potent biocidal effect than the majority of antibiotics used to treat Gram-negative bacteria. In addition, it was mentioned that the composite retains some physicochemical properties, like low adhesion, tensile strength, and water uptake [169]. BC is also useful as a material for treating various cancers thanks to its unique drug-loading and liquid-absorbing properties. BC-based scaffolds have been developed and analyzed to mimic tumor micro-environments for in-vitro culturing of cells. These scaffolds are primarily used by ovarian and breast cancer cells to maintain their normal proliferation. Proliferation, adhesion, ingrowth, and differentiation are all very noticeable [170]. In addition, incorporating additional features and methods utilizing water and tensile strength can increase BC's efficiency in drug delivery and produce better results [171]. Carbonization has also been used to impart chemical tolerance and improved conductivity upon BC without disrupting its characteristic 3-D structure. Enhanced volumetric resilience, ultra-low weight, super hydrophobicity, and a large surface area are just a few of the notable properties of carbonized BC (CBC) that are important for selective absorption and electrochemical energy storage [172]. In addition, a recent study focused on the thermal stability of BC found that the type of reinforcement material, such as nano-clays, ceramics, and metal oxides, significantly affects thermal stability. The extraordinary crystalline plane of these reinforcement materials reveals an outstanding increase in temperature stability, primarily by triggering chemical anaerobic reactions [173].

POTENTIAL OUTCOMES

Several novel nanomaterial's composite synthesis methods have brought into focuses the promising prospects and applications of bacterial cellulose. Better BC production can be achieved through the isolation of transcriptional factors, the discovery of novel microbial strains, the synthesis of engineered strains, and the synthesis of other regulatory elements that control the synthesis of BC. In addition, the morphology and shape of NPs materials including pressure, temperature, PH, and time must be optimized for their use in composites. These considerations are also important to keep in mind when trying to achieve a specific result. The creation of new cellulose-based functional materials for its use in BC-based composites also necessitates the development of a wide variety of complementary approaches. The

formation of a hydrogen-bonded network between the cellulose fibrils enables the regulation of the performance and adaptability of BC-based functional materials. It can be useful for learning more about how different fibers and materials functional groups are able to communicate with one another. Changing BC's structure to better interact with living tissues has the potential to advance the development of biomedical devices and synthetic organs. By enhancing the attachment of biological molecules like enzymes, protein cofactors, vaccines, antibodies, and some other drugs, it is potential for use in biomedicine can be greatly expanded.

Moreover, regardless of their potential biomedical applications, the full implications of silver nanoparticles (AgNPs) on human health must be clarified before they are used widely. However, advances in environmentally friendly, cost-effective, safe, and simple ways for the preparations of AgNPs are still required for their effective application in clinical settings using silver nanotechnology. Also, thoroughly researching and comprehending the mechanisms for control safety. Additionally, it is important to implement a suitable sterilization method because unsterile wound dressings and surgical implants introduce substantial levels of contamination. So, BC materials might be something to think about. Problems with drug release modulation and inconsistent pharmaceutical loading patterns are two more challenges that BC faces as a drug delivery agent. The widespread application of bacterial cellulose as a biomedical material in the coming years may be hampered without the discovery of novel and innovative processing techniques and the development of protocols to address these concerns.

CONCLUSIONS

This article describes the benefits of bio-nanocomposites made from bacterial cellulose (BC). Hydrogel composites based on BC, a promising biopolymer, have a wide range of potential uses in the medical field. In biomedicine, for example, the discovery of new strains of cellulose-producing bacteria could shed light on novel features that would allow for its application in more exciting areas like the treatment of various diseases, delivery of drugs, healing of wounds, formation of blood vessels, regeneration of tissue, development of biosensors, etc. Because BC is the purest form of cellulose and has superior properties to PC, such as higher porosity and biocompatibility, it needs to be studied further so that it can be used in new research areas and put to rest the problems that have arisen from its widespread use in biomedical applications.

REFERENCES

1. Esa, Faezah, Siti Masrinda Tasirin, and Norliza Abd Rahman. (2014). "Overview of bacterial cellulose production and application." Agriculture and Agricultural Science Procedia 2; 113-119.
2. Saibuatong, Ong-ard, and Muenduen Phisalaphong. (2010). "Novo aloe vera–bacterial cellulose composite film from biosynthesis." Carbohydrate Polymers 79.2: 455-460
3. Sun, Run-Cang. "Detoxification and separation of lignocellulose biomass prior to fermentation for bioEthanol production by removal of lignin and hemicelluloses." Bio Resources 4.2 (2009): 452-455.
4. Park, Joong Kon, Youn Hee Park, and Jae Yong Jung. "Production of bacterial cellulose by Gluconacetobacter hansenii PJK isolated from rotten apple." Biotechnology and Bioprocess Engineering 8.2 (2003): 83-88.
5. Lin, Wen-Chun, et al. (2013). "Bacterial cellulose and bacterial cellulose–chitosan membranes for wound dressing applications." Carbohydrate polymers 94.1 : 603-611
6. Seddiqi, H., Oliaei, E., Honarkar, H. et al.(2021). Cellulose and its derivatives: towards biomedical applications. Cellulose .11-16
7. Ullah, Muhammad Wajid, et al. (2021). "Recent developments in the synthesis, properties, and applications of various microbial polysaccharides." Handbook of Hydrocolloids. Woodhead Publishing, 975-1015.
8. Shi, Zhijun, et al. (2014). "Utilization of bacterial cellulose in food." Food hydrocolloids 35: 539-545.
9. Chen, Peng, Se Youn Cho, and Hyoung-Joon Jin. (2010). "Modification and applications of bacterial celluloses in polymer science." Macromolecular Research 18.4: 309-320.
10. Ul-Islam, Mazhar, et al. (2021). "Bacterial cellulose: trends in synthesis, characterization, and applications." Handbook of Hydrocolloids. Woodhead Publishing, 923-974.
11. Castro, Cristina, et al. (2011). "Structural characterization of bacterial cellulose produced by Gluconacetobacter swingsii sp. from Colombian agroindustrial wastes." Carbohydrate Polymers 84.1 : 96-102.
12. Keshk, S. M.(2014). "Bacterial cellulose production and its industrial applications." J Bioprocess Biotech 4.150: 2.90
13. Naomi, Ruth, Ruszymah Bt Hj Idrus, and Mh Busra Fauzi. (2020). "Plant-vs. Bacterial-derived cellulose for wound healing: A review." International journal of environmental research and public health 17.18 : 6803.
14. Ahmed, Enas M. (2015). "Hydrogel: Preparation, characterization, and applications: A review." Journal of advanced research 6.2: 105-121.
15. Lina, Fu, et al. (2011). "Bacterial cellulose for skin repair materials." Biomedical Engineering Frontiers and Challenges : 249-274.

16. Gullo, Maria, et al. (2019). "Exploring K2G30 genome: a high bacterial cellulose producing strain in glucose and mannitol based media." *Frontiers in microbiology* 10: 58.
17. McNamara, Karrina, and Syed AM Tofail. (2017). "Nanoparticles in biomedical applications." Advances in Physics: X 2.1: 54-88.
18. Gutierrez, Junkal, et al. (2013). "Multifunctional hybrid nanopapers based on bacterial cellulose and sol–gel synthesized titanium/vanadium oxide nanoparticles." Cellulose 20.3: 1301-1311.
19. Jindal, Namita, and Jasvirinder Singh Khattar. (2018). "Microbial polysaccharides in food industry." *Biopolymers for food design*. Academic Press, 95-123.
20. Ravella, S. R., Qui˜nones, T. S. R., Retter, A., Heiermann, M., Amon, T., & Hobbs, P.J. (2010). Extracellular polysaccharide (EPS) production by a novel strain of –like.
21. Sutherland, I. W. (1972). "Bacterial exopolysaccharides." Advances in microbial physiology 8: 143-213.
22. Branda, Steven S., et al.(2005). "Biofilms: the matrix revisited." Trends in microbiology 13.1 : 20-26.
23. Nichols, CA Mancuso, Jean Guezennec, and J. P. Bowman. (2005). "Bacterial exopolysaccharides from extreme marine environments with special consideration of the southern ocean, sea ice, and deep-sea hydrothermal vents: a review." Marine biotechnology 7.4: 253-271.
24. Dave, S. R., et al. "Microbial exopolysaccharide—An inevitable product for living beings and environment." J. Bacteriol. Mycol 2.4 (2016): 112.
25. Flemming, Hans-Curt, and Jost Wingender. (2010). "The biofilm matrix." Nature reviews microbiology 8.9: 623-633.
26. d'Abzac, Paul, et al. (2013). "Metal binding properties of extracellular polymeric substances extracted from anaerobic granular sludges." Environmental Science and Pollution Research 20.7: 4509-4519.
27. Olsen, I. (2015). "Biofilm-specific antibiotic tolerance and resistance." European Journal of Clinical Microbiology & Infectious Diseases 34.5: 877-886.
28. Rana, Sonali, and Lata Sheo Bachan Upadhyay.(2020). "Microbial exopolysaccharides: Synthesis pathways, types and their commercial applications." International journal of biological macromolecules 157: 577-583.
29. De Paniagua Michel, Jesús José, Jorge Olmos-Soto, and Eduardo Roberto Morales-Guerrero. (2014). "Algal and microbial exopolysaccharides: new insights as biosurfactants and bioemulsifiers." Advances in food and nutrition research 73: 221-257.
30. Donot, F., et al. (2012). "Microbial exopolysaccharides: main examples of synthesis, excretion, genetics and extraction." Carbohydrate Polymers 87.2 : 951-962.
31. Osemwegie, Osarenkhoe Omorefosa, et al. (2020). "Exopolysaccharides from bacteria and fungi: current status and perspectives in Africa." Heliyon 6.6: e04205.
32. Finore, Ilaria, et al. (2014). "Fermentation technologies for the optimization of marine microbial exopolysaccharide production." Marine drugs 12.5 : 3005-3024.
33. Zhang, Lixia, Wu, Xiaotong, et al. (2012). "Factors affecting extracellular and intracellular polysaccharide production in submerged cultivation of Tricholoma mongolicum." *African Journal of Microbiology Research* 6.5 (2012): 909-916.
34. Wu, Xiaotong, et al. (2012). "Factors affecting extracellular and intracellular polysaccharide production in submerged cultivation of Tricholoma mongolicum." African Journal of Microbiology Research 6.5: 909-916.
35. Czaczyk, Kasia, and Kamila Myszka. (2007). "Biosynthesis of extracellular polymeric substances (EPS) and its role in microbial biofilm formation." Polish Journal of Environmental Studies 16.6:90-98.
36. Kumar, Muthusamy Ashok, Kanapathi Thangavel Kasirajan Anandapandian, and Karuppiah Parthiban. (2011). "Production and characterization of exopolysaccharides (EPS) from biofilm forming marine bacterium." Brazilian archives of biology and technology 54; 259-265.
37. Tayeb, A.; Amini, E.; Ghasemi, S.; Tajvidi, M. (2018). Cellulose Nanomaterials—Binding Properties and Applications:A Review. Molecules 23, 2684.
38. Ullah, Hanif, et al.(2017). "Fabrication, characterization and evaluation of bacterial cellulose-based capsule shells for oral drug delivery." Cellulose 24.3: 1445-1454.
39. Vergara, B. S., P. M. H. Idowu, and J. H. Sumangil. (1999). "Nata de Coco–a Filipino delicacy." National Academy of Science and Technology. Metro Manila, Philippines: Island Publishing House, Philippines, Bicutan.
40. Gorgieva, Selestina.(2020). "Bacterial cellulose as a versatile platform for research and development of biomedical materials." Processes 8.5: 624.
41. Abol-Fotouh, Deyaa, et al. (2020). "Bacterial nanocellulose from agro-industrial wastes: low-cost and enhanced production by Komagataeibacter saccharivorans MD1." Scientific reports 10.1: 1-14.
42. Blanco Parte, Francisco German, et al. (2020). "Current progress on the production, modification, and applications of bacterial cellulose." Critical reviews in biotechnology 40.3: 397-414.
43. Kim, Yeji, et al. (2019). "Self-assembly of bio-cellulose nanofibrils through intermediate phase in a cell-free enzyme system." Biochemical Engineering Journal 142: 135-144.
44. Ullah, Muhammad Wajid, et al. (2016). "Structural and physico-mechanical characterization of bio-cellulose produced by a cell-free system." Carbohydrate polymers 136: 908-916.
45. Islam, M. S., et al. (2018). "Cellulose nanocrystal (CNC)–inorganic hybrid systems: synthesis, properties and applications." Journal of Materials Chemistry B 6.6 : 864-883.
46. Choi, Soon Mo, and Eun Joo Shin.(2020). "The nanofication and functionalization of bacterial cellulose and its applications." Nanomaterials 10.3 : 406.

47. Dahman, Yaser. (2009). "Nanostructured biomaterials and biocomposites from bacterial cellulose nanofibers." Journal of Nanoscience and Nanotechnology 9.9: 5105-5122.
48. Zhai, Xichuan, et al. (2020). "Improved characterization of nanofibers from bacterial cellulose and its potential application in fresh-cut apples." International journal of biological macromolecules 149: 178-186.
49. R. Rebelo, Ana, et al. (2018). "Dehydration of bacterial cellulose and the water content effects on its viscoelastic and electrochemical properties." *Science and Technology of advanced MaTerialS* 19.1: 203-211.
50. Ullah, Muhammad Wajid, et al. (2015). "Innovative production of bio-cellulose using a cell-free system derived from a single cell line." Carbohydrate polymers 132: 286-294.
51. McManus, John B., et al. (2016). "AcsA–AcsB: The core of the cellulose synthase complex from Gluconacetobacter hansenii ATCC23769." Enzyme and microbial technology 82: 58-65.
52. Lee, Koon-Yang, et al. (2014). "More than meets the eye in bacterial cellulose: biosynthesis, bioprocessing, and applications in advanced fiber composites." Macromolecular bioscience 14.1 10-32.
53. Tajima, Kenji, et al. (2021). "Cellulose-synthesizing machinery in bacteria." Cellulose: 1-23.
54. Jacek, Paulina, et al. (2019). "Molecular aspects of bacterial nanocellulose biosynthesis." Microbial biotechnology 12.4 : 633-649.
55. Morgan, Jacob LW, Joanna Strumillo, and Jochen Zimmer.(2013). "Crystallographic snapshot of cellulose synthesis and membrane translocation." Nature 493.7431 : 181-186.
56. Endler, Anne, Clara Sánchez-Rodríguez, and Staffan Persson. "Cellulose squeezes through." Nature chemical biology 6.12 (2010): 883-884.
57. Omadjela, Okako, et al.(2013). "BcsA and BcsB form the catalytically active core of bacterial cellulose synthase sufficient for in vitro cellulose synthesis." *Proceedings of the National Academy of Sciences* 110.44: 17856-17861.
58. Saxena, Inder M., et al. (1994). "Characterization of genes in the cellulose-synthesizing operon (acs operon) of Acetobacter xylinum: implications for cellulose crystallization." Journal of bacteriology 176.18: 5735-5752.
59. Buldum, Gizem, Alexander Bismarck, and Athanasios Mantalaris. (2018). "Recombinant biosynthesis of bacterial cellulose in genetically modified Escherichia coli." *Bioprocess and biosystems engineering* 41.2: 265-279.
60. Hu, S. -Q., Gao, Y. -G., Tajima, K., Sunagawa, N., Zhou, Y., Kawano, S.,…Yao, M. (2010). Structure of bacterial cellulose synthase subunit D octamer with four inner passageways. Proceedings of the National Academy of Sciences, 107, 17957–17961. https://doi.org/10.1073/ pnas.1000601107.
61. Mehta, K., Pfeffer, S., & Brown, R. M. (2015). Characterization of an acsD disruption mutant provides additional evidence for the hierarchical cell-directed self-assembly of cellulose in Gluconacetobacter xylinus. Cellulose, 22, 119–137
62. Sunagawa, Naoki, et al. "Cellulose complementing factor (Ccp) is a new member of the cellulose synthase complex (terminal complex) in Acetobacter xylinum." *Journal of bioscience and bioengineering* 115.6 (2013): 607-612.
63. Buldum, Gizem. "Investigation of bacterial cellulose production in genetically modified Escherichia coli." (2015).
64. Nakai, T., Sugano, Y., Shoda, M., Sakakibara, H., Oiwa, K., Tuzi, S.,…Mineyukia, Y. (2013). Formation of highly twisted ribbons in a carboxymethylcellulase gene-disrupted strain of a cellulose producing bacterium. Journal of Bacteriology.
65. Sunagawa, Naoki, et al. "Cellulose production by Enterobacter sp. CJF-002 and identification of genes for cellulose biosynthesis." *Cellulose* 19.6 (2012): 1989-2001.
66. Shah, Nasrullah, et al. "Overview of bacterial cellulose composites: a multipurpose advanced material." Carbohydrate polymers 98.2 (2013): 1585-1598.
67. Shezad, Omer, et al. "Physicochemical and mechanical characterization of bacterial cellulose produced with an excellent productivity in static conditions using a simple fed-batch cultivation strategy." Carbohydrate Polymers 82.1 (2010): 173-180.
68. Wang, Jing, Javad Tavakoli, and Youhong Tang. (2019). "Bacterial cellulose production, properties and applications with different culture methods–A review." Carbohydrate polymers 219: 63-76.
69. Adnan, Azila, et al. (2021). "Bacterial Cellulose Synthesis by Gluconacetobacter xylinus: Enhancement via Fed-batch Fermentation Strategies in Glycerol Media." *Trends in Sciences* 18.22: 453-453.
70. Fatima, Atiya, et al. (2021). "Plant extract-loaded bacterial cellulose composite membrane for potential biomedical applications." Journal of Bioresources and Bioproducts 6.1: 26-32.
71. Liu, Wei, et al. (2020). "Bacterial cellulose-based composite scaffolds for biomedical applications: a review." ACS Sustainable Chemistry & Engineering 8.20: 7536-7562.
72. Gao, Minghong, et al. (2019). "A natural in situ fabrication method of functional bacterial cellulose using a microorganism." Nature communications 10.1: 1-10.
73. Ul-Islam, Mazhar, Taous Khan, and Joong Kon Park.(2012). "Nanoreinforced bacterial cellulose–montmorillonite composites for biomedical applications." Carbohydrate polymers 89.4: 1189-1197.
74. Khaledian, Yousef, Mohammadreza Pajohi-Alamoti, and Behnaz Bazargani-Gilani. (2019). "Development of cellulose nanofibers coating incorporated with ginger essential oil and citric acid to extend the shelf life of ready-to-cook barbecue chicken." Journal of Food Processing and Preservation 43.10: e14114.
75. Yin, Na, et al. (2014). "Bacterial cellulose as a substrate for microbial cell culture." Applied and environmental microbiology 80.6: 1926-1932.
76. Tang, Weihua, et al. (2010). "The influence of fermentation conditions and post-treatment methods on porosity of bacterial cellulose membrane." World Journal of Microbiology and Biotechnology 26.1: 125-131.

77. Torres, Fernando G., et al.(2009). "Reversible stress softening and stress recovery of cellulose networks." Soft Matter 5.21: 4185-4190.
78. Cai, Yi, et al. (2017). "Walnut-like porous core/shell TiO2 with hybridized phases enabling fast and stable lithium storage." ACS applied materials & interfaces 9.12: 10652-10663.
79. Pandey, Ashok, et al., eds. (2015). *Industrial biorefineries and white biotechnology*. Elsevier.
80. Grangeteau, Cédric, et al. (2016). "FT-IR spectroscopy: A powerful tool for studying the inter-and intraspecific biodiversity of cultivable non-Saccharomyces yeasts isolated from grape must." Journal of microbiological methods 121: 50-58.
81. Bunaciu, Andrei A., Elena Gabriela UdriŞTioiu, and Hassan Y. Aboul-Enein. (2015). "X-ray diffraction: instrumentation and applications." Critical reviews in analytical chemistry 45.4: 289-299.
82. Karim, Zoheb, et al. (2014). "Nanoporous membranes with cellulose nanocrystals as functional entity in chitosan: removal of dyes from water." Carbohydrate polymers 112: 668-676.
83. Ullah, Muhammad Wajid, et al. (2016). "In situ synthesis of a bio-cellulose/titanium dioxide nanocomposite by using a cell-free system." RSC advances 6.27: 22424-22435.
84. Roy, Joyita, and Kunal Roy. (2012). "Assessment of toxicity of metal oxide and hydroxide nanoparticles using the QSAR modeling approach." *Environmental Science: Nano* 8.11: 3395-3407.
85. Cho, Eun Jung, et al. (2013). "Nanoparticle characterization: state of the art, challenges, and emerging technologies." Molecular pharmaceutics 10.6: 2093-2110.
86. Machado, S., et al. (2015). "Characterization of green zero-valent iron nanoparticles produced with tree leaf extracts." Science of the total environment 533: 76-81.
87. Scarcelli, Vittoria, et al. "Patrizia Guidi, Margherita Bernardeschi, Mara Palumbo, Massimo Genovese .(2021). " *Nanotechnology for Environmental and Biomedical Research* (2021): 23.
88. Shin, Won-Kyung, et al. (2016). "Cross-linked composite gel polymer electrolyte using mesoporous methacrylate-functionalized SiO 2 nanoparticles for lithium-ion polymer batteries." Scientific reports 6.1: 1-10.
89. Lee, Ji Eun, et al. (2011). "Multifunctional mesoporous silica nanocomposite nanoparticles for theranostic applications." Accounts of chemical research 44.10; 893-902.
90. Rawal, Ishpal, and Amarjeet Kaur. (2013). "Synthesis of mesoporous polypyrrole nanowires/nanoparticles for ammonia gas sensing application." Sensors and Actuators A: Physical 203: 92-102
91. Muhammad, Shaima Farhad. (2021). Nanotechnology Particle characteristic. Diss. Salahaddin University-Erbil.
92. Oprică, Lacrămioara, and Maria Bălăşoiu. (2019). "Nanoparticles: an overview about their clasifications, synthesis, properties, characterization and applications." Journal of Experimental and Molecular Biology 20.4: 43-60.
93. Ealia, S. Anu Mary, and M. P. Saravanakumar. (2017). "A review on the classification, characterisation, synthesis of nanoparticles and their application." *IOP Conference Series: Materials Science and Engineering*. Vol. 263. No. 3. IOP Publishing.
94. Lee, Sang Hun, Jong Hwan Sung, and Tai Hyun Park. (2012). "Nanomaterial-based biosensor as an emerging tool for biomedical applications." Annals of biomedical engineering 40.6: 1384-1397.
95. Alshehri, Ali H., et al. "Enhanced electrical conductivity of silver nanoparticles for high frequency electronic applications." ACS applied materials & interfaces 4.12 (2012): 7007-7010.
96. Chen, Guofang, et al. (2014). "A novel green synthesis approach for polymer nanocomposites decorated with silver nanoparticles and their antibacterial activity." Analyst 139.22: 5793-5799.
97. Wang, Liming, et al. (2015). "Use of synchrotron radiation-analytical techniques to reveal chemical origin of silver-nanoparticle cytotoxicity." ACS nano 9.6 : 6532-6547.
98. Nene, Ajinkya Girish, et al. (2021). "Synthetic preparations and atomic scale engineering of silver nanoparticles for biomedical applications." *Nanoscale* :9-15.
99. Khan, Ibrahim, Khalid Saeed, and Idrees Khan. (2019). "Nanoparticles: Properties, applications and toxicities." Arabian journal of chemistry 12.7: 908-931.
100. Emeji, Ikenna Chibuzor, et al.(2020)."Properties and Synthesis of Metal Oxide Nanoparticles in Electrochemistry." Nanostructured Metal-Oxide Electrode Materials for Water Purification. Springer, Cham, 85-96.
101. Vázquez, Ester, Francesco Giacalone, and Maurizio Prato.(2014). "Non-conventional methods and media for the activation and manipulation of carbon nanoforms." Chemical Society Reviews 43.1 : 58-69.
102. La Mantia, F. P., and M. Morreale. (2011). "Green composites: A brief review." Composites Part A: Applied Science and Manufacturing 42.6: 579-588.
103. Sanjay, M. R., et al. (2018). "Characterization and properties of natural fiber polymer composites: A comprehensive review." Journal of Cleaner Production 172 : 566-581.
104. Deng, Yelin, et al. (2016). "Life cycle assessment of flax-fibre reinforced epoxidized linseed oil composite with a flame retardant for electronic applications." Journal of Cleaner Production 133: 427-438.
105. Sharma, Ruchi, Seid Mahdi Jafari, and Somesh Sharma. (2020). "Antimicrobial bio-nanocomposites and their potential applications in food packaging." Food Control 112: 107086.
106. Shchipunov, Yury. (2012)."Bionanocomposites: Green sustainable materials for the near future." Pure and Applied Chemistry 84.12 : 2579-2607.
107. Wagner, M. P. (1941). Rubber World, 164, p. 46 book

108. Zafar, Rabia, et al. (2016). "Polysaccharide based bionanocomposites, properties and applications: A review." International journal of biological macromolecules 92 : 1012-1024.
109. Ul-Islam, Mazhar, et al. (2015). "Bacterial cellulose composites: Synthetic strategies and multiple applications in bio-medical and electro-conductive fields." Biotechnology journal 10.12: 1847-1861.
110. Volova, Tatiana G., et al. (2018). "Antibacterial properties of films of cellulose composites with silver nanoparticles and antibiotics." Polymer Testing 65: 54-68.
111. Chang, Wen-Shuo, and Hui-Huang Chen. (2016). "Physical properties of bacterial cellulose composites for wound dressings." Food Hydrocolloids 53: 75-83.
112. Feng, Yiyu, et al. (2012). "A mechanically strong, flexible and conductive film based on bacterial cellulose/graphene nanocomposite." Carbohydrate Polymers 87.1 : 644-649.
113. Eslahi, Niloofar, et al. (2020). "Processing and properties of nanofibrous bacterial cellulose-containing polymer composites: a review of recent advances for biomedical applications." Polymer Reviews 60.1: 144-170.
114. Simon, Juliette, (2019). Emmanuel Flahaut, and Muriel Golzio. "Overview of carbon nanotubes for biomedical applications." Materials 12.4: 624.
115. Zhang, Taiji, et al. (2010). "Biotemplated synthesis of gold nanoparticle–bacteria cellulose nanofiber nanocomposites and their application in biosensing." Advanced Functional Materials 20.7: 1152-1160.
116. Ul-Islam, Mazhar, et al. (2013). "Effect of post-synthetic processing conditions on structural variations and applications of bacterial cellulose." Cellulose 20.1: 253-263.
117. Torres, Fernando G., Solene Commeaux, and Omar P. Troncoso. (2012). "Biocompatibility of bacterial cellulose based biomaterials." Journal of Functional Biomaterials 3.4 : 864-878.
118. Shi, Zhijun, et al.(2012). "In situ nano-assembly of bacterial cellulose–polyaniline composites." Rsc Advances 2.3 : 1040-1046.
119. Kim, Jaehwan, et al. (2011). "Preparation and characterization of a bacterial cellulose/chitosan composite for potential biomedical application." Journal of Polymer Research 18.4: 739-744.
120. Ul-Islam, Mazhar, et al. (2013). "Effects of glucuronic acid oligomers on the production, structure and properties of bacterial cellulose." Carbohydrate polymers 92.1 : 360-366.
121. Cheng, Kuan-Chen, Jeff M. Catchmark, and Ali Demirci. (2009). "Enhanced production of bacterial cellulose by using a biofilm reactor and its material property analysis." Journal of biological engineering 3.1: 1-10.
122. Ul-Islam, Mazhar, et al. (2011). "Effect of chitosan penetration on physico-chemical and mechanical properties of bacterial cellulose." Korean Journal of Chemical Engineering 28.8 : 1736-1743.
123. Ullah, Muhammad Wajid, et al. (2019). "Synthesis, structure, and properties of bacterial cellulose." *Nanocellulose: From Fundamentals to Advanced Materials* : 81-113.
124. Moniri, Mona, et al. (2017). "Production and status of bacterial cellulose in biomedical engineering." Nanomaterials 7.9 : 257.
125. Khan, Shaukat, et al. (2015). "Bacterial cellulose-titanium dioxide nanocomposites: nanostructural characteristics, antibacterial mechanism, and biocompatibility." Cellulose 22.1: 565-579.
126. Sabbagh, Farzaneh, Ida Idayu Muhamad, Norhayati Pa'e, and Zanariah Hashim. (2019). "Strategies in improving properties of cellulose-based hydrogels for smart applications." Cellulose-Based Superabsorbent Hydrogels: 887-908.
127. Ma, Jianzhong, Xiaolu Li, and Yan Bao.(2015). "Advances in cellulose-based superabsorbent hydrogels." RSC advances 5.73 : 59745-59757.
128. Wahid, Fazli, et al. (2020). "Nanocomposite hydrogels as multifunctional systems for biomedical applications: Current state and perspectives." Composites Part B: Engineering: 108208.
129. Biondi, Marco, et al. "Nanoparticle-integrated hydrogels as multifunctional composite materials for biomedical applications." *Gels* 1.2 (2015): 162-178.
130. Czaja, Wojciech K., et al. "The future prospects of microbial cellulose in biomedical applications." biomacromolecules 8.1 (2007): 1-12.
131. Peck, Michael D. "Epidemiology of burns throughout the world. Part I: Distribution and risk factors." Burns 37.7 (2011): 1087-1100.
132. Khalid, Ayesha, et al. "Bacterial cellulose-zinc oxide nanocomposites as a novel dressing system for burn wounds." Carbohydrate polymers 164 (2017): 214-221.
133. Barud, Hernane S., et al. "Antimicrobial bacterial cellulose-silver nanoparticles composite membranes." Journal of Nanomaterials 2011 (2011).
134. Herndon, David N., et al. (1989). "A comparison of conservative versus early excision. Therapies in severely burned patients." *Annals of surgery* 209.5 : 547.
135. Lamboni, Lallepak, et al.(2016). "Silk sericin-functionalized bacterial cellulose as a potential wound-healing biomaterial." Biomacromolecules 17.9: 3076-3084.
136. Gorgieva, Selestina, and Janja Trček.(2019). "Bacterial cellulose: Production, modification and perspectives in biomedical applications." Nanomaterials 9.10 : 1352.
137. Wu, Zhuotong, et al. (2020). "Top-down peeling bacterial cellulose to high strength ultrathin films and multifunctional Engineering fibers." Chemical Journal 391: 123527.
138. Atiyeh, Bishara S., et al.(2007). "Effect of silver on burn wound infection control and healing: review of the literature." burns 33.2: 139-148.

139. Swingler, Sam, et al. (2021)."The Mould War: Developing an Armamentarium against Fungal Pathogens Utilising Thymoquinone, Ocimene, and Miramistin within Bacterial Cellulose Matrices." *Materials* 14.10: 2654.
140. Marestoni, Luiz Diego, et al. (2021). "Commercial and potential applications of bacterial cellulose in Brazil: ten years review." Polímeros 30 :09-14.
141. Qiu, Yuyu, et al. (2016). "Bacterial cellulose and bacterial cellulose-vaccarin membranes for wound healing." Materials Science and Engineering: C 59;: 303-309.
142. Mohite, Bhavna V., and Satish V. Patil. (2014). "A novel biomaterial: bacterial cellulose and its new era applications." Biotechnology and Applied Biochemistry 61.2: 101-110.
143. Picheth, Guilherme Fadel, et al. "Bacterial cellulose in biomedical applications: A review." *International journal of biological macromolecules* 104 (2017): 97-106.
144. Wei, Bin, Guang Yang, and Feng Hong. "Preparation and evaluation of a kind of bacterial cellulose dry films with antibacterial properties." *Carbohydrate Polymers* 84.1 (2011): 533-538.
145. Ahmed, Jubair, Merve Gultekinoglu, and Mohan Edirisinghe. "Bacterial cellulose micro-nano fibres for wound healing applications." Biotechnology advances 41 (2020): 107549.
146. Rezvani Ghomi, Erfan, et al. (2019). "Wound dressings: Current advances and future directions." *Journal of Applied Polymer Science* 136.27: 47738.
147. Mbituyimana, Bricard, et al. (2021). "Bacterial cellulose-based composites for biomedical and cosmetic applications: Research progress and existing products." *Carbohydrate Polymers* 273 : 118565.
148. Oprea, Madalina, and Denis Mihaela Panaitescu.(2020). "Nanocellulose hybrids with metal oxides nanoparticles for biomedical applications." Molecules 25.18: 4045.
149. Akhtar, Mohd Javed, et al. (2012). "Zinc oxide nanoparticles selectively induce apoptosis in human cancer cells through reactive oxygen species." International journal of nanomedicine 7 ; 845.
150. Rajendra, Radhai, et al.(2010). "Use of zinc oxide nano particles for production of antimicrobial textiles." International Journal of Engineering, Science and Technology 2.1: 202-208.
151. Ul-Islam, Mazhar, et al. (2014). "Synthesis of regenerated bacterial cellulose-zinc oxide nanocomposite films for biomedical applications." Cellulose 21.1: 433-447.
152. Cai, Zhijiang, and Jaehwan Kim. (2010). "Bacterial cellulose/poly (ethylene glycol) composite: characterization and first evaluation of biocompatibility." Cellulose 17.1: 83-91.
153. Chandrasekhar, K., et al. (2020). "Sources of Natural Polymers from Microorganisms with Green Nanoparticles." *Green Polymeric Nanocomposites*. CRC Press, 103-132.
154. Bacakova, Lucie, et al. (2020). "Applications of nanocellulose/nanocarbon composites: Focus on biotechnology and medicine." Nanomaterials 10.2 : 196.
155. Sulaeva, Irina, et al. (2015). "Bacterial cellulose as a material for wound treatment: Properties and modifications. A review." Biotechnology advances 33.8: 1547-1571.
156. Peres, Maristela FS, et al. (2016). "Bacterial cellulose membranes as a potential drug delivery system for photodynamic therapy of skin cancer." Journal of the Brazilian Chemical Society 27 : 1949-1959.
157. Badshah, Munair, et al. (2018). "Surface modification and evaluation of bacterial cellulose for drug delivery." International journal of biological macromolecules 113: 526-533.
158. Plackett, David, et al. (2014). "A review of ncellulose as a novel vehicle for drug delivery." Nordic Pulp & Paper Research Journal 29.1: 105-11ano8.
159. Varaprasad, Kokkarachedu, et al.(2017). "A mini review on hydrogels classification and recent developments in miscellaneous applications." Materials Science and Engineering: C 79: 958-971.
160. Zagórska-Dziok, Martyna, and Marcin Sobczak. (2020). "Hydrogel-based active substance release systems for cosmetology and dermatology application: a review." *Pharmaceutics* 12.5: 396.
161. Yang, Yu-Ning, et al. "Development of bacterial cellulose/chitin multi-nanofibers based smart films containing natural active microspheres and nanoparticles formed in situ." Carbohydrate polymers 228 (2020): 115370
162. Mohite, BHAVANA V., RAHUL K. Suryawanshi, and SATISH V. Patil. "Study on the drug loading and release potential of bacterial cellulose." Cellulose Chem Technol 50 (2016): 219-223.
163. Shao, Wei, et al. (2016). "Controlled release and antibacterial activity of tetracycline hydrochloride-loaded bacterial cellulose composite membranes." Carbohydrate polymers 145 : 114-120.
164. Ojagh, Seyed Mohammad Amin, Farzaneh Vahabzadeh, and Afzal Karimi. (2021)."Synthesis and characterization of bacterial cellulose-based composites for drug delivery." *Carbohydrate Polymers* 273: 118587.
165. Huang, Lin, et al. (2013). "Nano-cellulose 3D-networks as controlled-release drug carriers." *Journal of Materials Chemistry B* 1.23: 2976-2984.
166. Gregory, David A., et al. (2021). "Bacterial cellulose: A smart biomaterial with diverse applications." Materials Science and Engineering: R: Reports 145: 100623.
167. Wichai, Siripan, et al. "Development of bacterial cellulose/alginate/chitosan composites incorporating copper (II) sulfate as an antibacterial wound dressing." Journal of Drug Delivery Science and Technology 51 (2019): 662-671.
168. Ma, Bo, et al.(2021). "Construction of silver nanoparticles anchored in carbonized bacterial cellulose with enhanced antibacterial properties." Colloids and Surfaces A: Physicochemical and Engineering Aspects 611: 125845.
169. Rubina, M. S., et al. (2019). "The interaction effect of bacterial cellulose with gold nanoparticles obtained by metal-vapor synthesis.". Vol. 488. No. 4. 100-109

170. Islam, Salman Ul, et al. (2021). "Potential applications of bacterial cellulose and its composites for cancer treatment." International Journal of Biological Macromolecules 168: 301-309.
171. Gupta, Abhishek, et al. (2020). "Synthesis of silver nanoparticles using curcumin-cyclodextrins loaded into bacterial cellulose-based hydrogels for wound dressing applications." Biomacromolecules 21.5: 1802-1811.
172. Huang, Yang, et al. (2021). "Recent advances on the bacterial cellulose-derived carbon aerogels." Journal of Materials Chemistry C 9.3: 818-828.
173. Torgbo, Selorm, and Prakit Sukyai. (2020). "Biodegradation and thermal stability of bacterial cellulose as biomaterial: The relevance in biomedical applications." Polymer Degradation and Stability 179: 109232.