

**Review Article**

Structure, Properties and Medical Advances for Biocellulose Applications: A Review

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Abstract: Microbial exopolysaccharides (EPS) obtained from microbial sources, became simply available for a broad range of applications, especially for medicine and pharmaceutical industries. One of these EPS bacterial cellulose known as biocellulose (BC). BC is a pure extracellular cellulose, which accumulate outside the cells, produced by several species of microorganisms, like *Gluconacetobacter*; *Achromobacter*; *Sarcina* and *Agrobacterium* with a great number of applications. It is an organic unbranched polysaccharide, type β -1,4-glucan, composed of glucopyranose residues. Biocellulose is used as artificial skin and occlusive dressings to treat chronic wounds and to heal burns, in microsurgeries as artificial blood vessels, scaffolds in tissue engineering and many other applications. This work represents a review on this remarkably microbial biomaterial and thus an update of a collection of scientific data from original research between 1979 and 2016. The paper started on structural, composition, properties and characterization and biogenesis of this biopolymer, then it gives a synthesis on a variety of biomedical applications.

Keywords: Biopolymer, Biocellulose, Biogenesis, *Gluconacetobacter*

1. Introduction

Cellulose is the majority abundant natural polymer on earth, documented as the main component of plant biomass, but also representative of microbial extracellular polymer. Bacterial cellulose (BC) or microbial cellulose (MC), called biocellulose (BC), is an organic polymer produced by the culture of a range of kinds of bacteria and it was first recognized as cellulose in 1886, it is composed of glucose monomers, with chemical formula $(C_6H_{10}O_5)_n$, formed, particularly by *Acetobacter xylinum*, a gram negative bacterium, which can produce cellulose and acetic acid during growth and release them into the environment [1-5]. This species can synthesis enough cellulose for commercial purposes. There are also other bacteria that can make bacterial cellulose with genus *Aerobacter*; *Achromobacter*; *Agrobacterium*, *Alcaligenes*, *Zoogloea*, *Pseudomonas*, *Rhizobium* and *Sarcina*. These bacteria fashioned biocellulose in different form. *Acetobacter* produced cellulose in the form of ribbons, *Aerobacter*; *Achromobacter* and

Alcaligenes in fibrils form, *Agrobacterium* and *Rhizobium* in the form of short fibril, *Pseudomonas* produce cellulose with no distinct fibril, *Sarcina* produce an amorphous cellulose and *Zoogloea* produces cellulose in not well defined form [1, 6].

One of the most important characteristics of biocellulose is its chemical purity, which distinguishes this cellulose from that from plants, usually associated with hemicelluloses and lignin, removal of which is inherently difficult, its ultra-fine network structure, high biodegradability and unique mechanical strength. Bacterial cellulose is one of the most promising basic biological material, with extensive application perspectives brings extraordinary economic benefits in different fields, such as food, textiles, paper, composite membranes, medicine, artificial skin and blood vessels, binders, diaphragms [1, 7-8].

This work present data concerning structure, physicochemical properties, synthesis of microbial cellulose and applications in medicine.

2. Structure and Properties

Cellulose is an unbranched polysaccharide of β 1,4-linked glucopyranose residues (figure 1). Extensive research on BC revealed that it is chemically identical to plant cellulose (PC), but its macromolecular structure and properties differ from the latter. Microfibrils of BC were about 100 times smaller than plant cellulose (figure 2) and the glucan chains are held together by inter- and intra-hydrogen bonding [1,9].

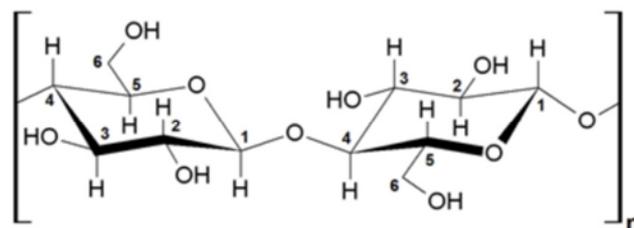


Figure 1. Representative structure of bacterial cellulose (BC) [10].

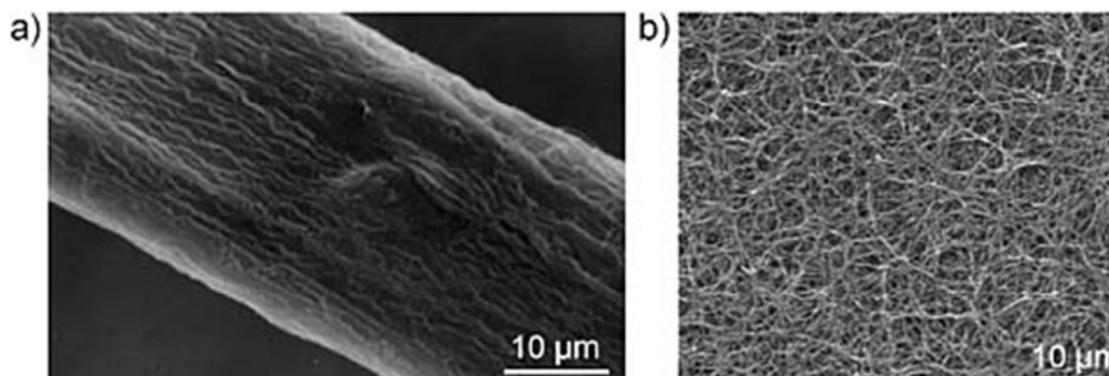


Figure 2. Microscopic observations of: a) plant cellulose; b) bacterial cellulose [11].

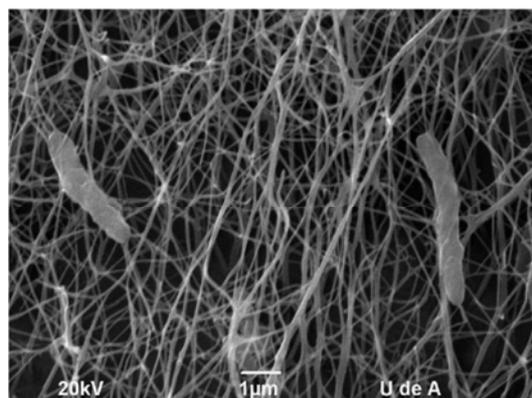


Figure 3. SEM image of a pellicle and bacteria (*Gluconacetobacter swingsii* sp.) with attached cellulose ribbons [18].

BC structures are formed by extracellularly-excreted nanofibers (about 20-100nm in diameter and around 100 μ m in length) and has a very high surface area per unit mass, produced by various species of bacteria, including *Acetobacter*, *Rhizobium*, *Agrobacterium* and *Sarcina*. The ribbons are made up of bundles of cellulose microfibrils of 2-4nm in diameter (figure 3). The average mesh size (distance between junction points) is $0.523 \pm 0.273 \mu\text{m}$, while the orientation (the average angle formed by the segments and the x-axis) of the nanofibers is $85.64 \pm 0.56^\circ$ [12-15]. One of the greatest advantages of microbial cellulose is its ability to be molded into almost any size and shape during its synthesis without causing any significant alteration of its physical properties. BC shows high tensile strength and modulus, due to high crystalline structure and reduced fiber diameter. For instance, the BC microfibrils have a density of 1600kg/m^3 , Young's modulus of 138GPa and tensile strength of at least 2GPa, which are almost equal to those of aramid fibers [14,

16-17]. Bacterial cellulose has a high purity where it is free from lignin, hemicelluloses and waxy aromatic substance than plant cellulose that usually associated with these materials where the removal is very difficult. Bacterial cellulose has high crystallinity, high water adsorption capacity, and high mechanical strength in wet state, ultrafine network structure, mouldability in situ and availability in an initial wet state [6].

Investigation of various cellulose samples by Nuclear Magnetic Resonance (NMR) spectroscopy found that all natural cellulose was a complex of both $I\alpha$ and $I\beta$ forms, and the content of $I\alpha$ was about 65% in BC. The ratio of cellulose $I\alpha$ and $I\beta$ differs greatly from species to species [13,19-21]. The study of Hirai et al. [22] showed that decreased $I\alpha$ -cellulose content led to smaller microfibrils in BC. Based on X-ray investigations, BC membranes exhibit uniplanar orientation and an additional axial orientational component that depends on the drying procedure, deformation of BC can be achieved by soaking the samples in NaOH solutions with concentration ranging from 8 to 10wt%. Treatment with NaOH had minimal effects on the mechanical properties [13].

Bacterial cellulose, which is produced by *Gluconacetobacter xylinus*, are found to be rich in $I\alpha$, the average mass fraction of cellulose $I\alpha$ is about 0.63. The primary difference between cellulose $I\alpha$ and $I\beta$ appears at the pattern of C1 resonance around 106ppm, singlet resonance for $I\alpha$ and doublet one for $I\beta$. The structure of cellulose $I\beta$ must be thermodynamically more stable than that of cellulose $I\alpha$. Cellulose $I\alpha$ is crystallize in larger-size microfibrils, whereas cellulose $I\beta$ is formed in smaller-size microfibrils. Cellulose $I\alpha$ must be crystallized in the higher energy state compared to the case of cellulose $I\beta$. The unit cell of cellulose $I\alpha$ is a triclinic and that for cellulose $I\beta$ is monoclinic unit cell [21, 23].

3. Microbial Cellulose Biosynthesis

Cellulose synthesis by microbial cells is a four-step process: (1) Activation of monosaccharides through formation of sugar nucleotides, (2) assembly of repeating units by sequential addition of sugar units through polymerization, (3) simultaneous addition of acyl groups (if present), and (4) excretion through the wall/membrane complex at the cell surface, into the extracellular environment[24]. The steps of biocellulose synthesis are regulated by a large number of individual enzymes and complexes of catalytic and regulatory proteins [1].

Gluconacetobacter xylinus is currently considered a model organism for the study these biopolymers. Formerly known as *Acetobacter xylinum*, this bacterium was reclassified and scientifically cataloged as *Gluconacetobacter xylinus*, due to the characteristics phylogeny based on analysis of partial sequences of 16S ribosomal RNA. It is a gram negative rod-shaped microorganisms with a length is in the range of 2 to 10 microns and a width in the range 0.5 to 1 micron [25-28].

This bacterium produce relatively high levels of polymer from wide range of carbon and nitrogen sources. Cellulose synthesized by *A. xylinum* is a final product of carbon metabolism, which depending on the physiological state of the cell involves either the pentose phosphate cycle or the krebs cycle, coupled with gluconeogenesis. Cellulose produced by *Acetobacter* strain was found to be chemically pure, free of lignin and hemicelluloses and to have different properties from wood derived cellulose. In *A. xylinum*, cellulose synthesis is tightly associated with catabolic processes of oxidation and consumes as much as 10% of energy derived from catabolic reactions. BC production does not interfere with other anabolic processes, including protein synthesis. The formation of biocellulose starts from the aggregation of glucan chain that consist of approximately 6-8 units are elongated from the complex. The formation of microfibril occur by assembling sub-elementary of this fibril and tighten the microfibril in order to form an interwoven ribbon [1, 6].

Production of MC by fermentation process require temperature, pH and nutrients. The maximal biocellulose production was observed between 28°C to 30°C and the optimum pH of the culture medium is in range of 4 and 6. The simple sugar such as glucose, xylose or other carbon source such as ethanol and glycerol are required. BC is still expensive compared with other popular commercial organic products of the usage of pure glucose as a substrate. Rather than pure glucose, wastes that contain glucose usually in small amount also can be use as a substrate such as corn steep liquor, fruit waste, fruit skin or state milk. *Acetobacter xylinum* can grow at a pH level between 3.5-7.5 (optimum at pH 4.3), the ideal temperature for the growth is 28°C-31°C. *Acetobacter xylinum* is aerobic and therefore need oxygen in its process of growth, development and activity. It has a resistance to sudden changes such as the decrease of water in a medium composition, pH, presence of toxic substances and pathogenic organisms [6, 27-28].

For the biogenesis, microorganisms produce cellulose

synthase complexes. The cellulose synthase protein is a β -glycosyltransferase. The X-ray crystallographic structure of cellulose synthase was reported from the minimally required subunits of bacterial cellulose synthase, CesaA and CesaB, of the purple bacterium *Rhodobacter sphaeroides*[29-30]. In *Acetobacter xylinum*, an operon encoding four proteins required for bacterial cellulose biosynthesis was isolated. Nucleotide sequence analysis indicated that the cellulose synthase operon is 9217 base pairs long and consists of four genes. The four genes, named -bcsA, bcsB, bcsC, and bcsD- appear to be translationally coupled and transcribed as a polycistronic mRNA with an initiation site 97 bases upstream of the coding region of the first gene (besA) in the operon. All four genes in the operon are required for maximal bacterial cellulose synthesis in *A. xylinum*. The calculated molecular masses of the proteins encoded by bcsA, bcsB, bcsC and bcsD are 84.4, 85.3, 141.0, and 17.3kDa, respectively [31].

The cellulose synthase machinery in *Gluconacetobacter hansenii* was observed as a Terminal Complex (TC) arranged in linear rows by freeze-fracture transmission electron microscopy and immunogold labeling. The first cellulose synthase gene to be identified was called acsA, and a number of studies have shown that the synthase works optimally when supplemented with the products of the genes, called acsB, acsC and acsD [31-35]. In *G. hansenii*, The catalytic subunit, AcsA (83kDa) exists as a complex with accessory protein AcsB (93kDa). The catalytic AcsAB complex is embedded in the cytoplasmic membrane. AcsA transfer glucose to the growing glucan chain [36-41]. The C-terminal portion of AcsC is predicted to form a translocation channel in the outer membrane, with the rest of AcsC possibly interacting with AcsD in the periplasm. It is thus believed that synthesis from an organized array of TCs coordinated with extrusion by AcsC and AcsD enable this bacterium to make crystalline cellulose [42].

MC synthesis by *G. xylinus* results of a metabolic pool hexose phosphate which is produced directly by phosphorylating exogenous hexose or indirectly by the pentose phosphate pathway and gluconeogenesis. The hexose phosphate conversion of cellulose is direct and does not depend on the intermediate divisions carbon skeleton. The conversion of glucose, transported from the external environment into the cytoplasm, is catalyzed by four bacterial enzymes, the glucokinase, which is the enzyme responsible for the phosphorylation of the carbon 6 of glucose, yielding glucose-6-phosphate, the phosphoglucomutase, which catalyzes the reaction isomerization of glucose-6-phosphate to glucose-1-phosphate, the UDPG-pyrophosphorylase (also known as glucose-1-phosphate uridylyltransferase), responsible for synthesis of UDP-glucose (UDPG), and cellulose synthase (CS), responsible for the polymerization of cellulose from UDP-glucose. In *G. xylinus*, the synthesis from endogenous sources begins with oxaloacetate, into pyruvate by action of the enzyme pyruvate carboxylase [34, 43-44].

In *A. xylinum*, the subunits catalytic of cellulose synthesis are organized in a linear fashion on the main axis of the cell. The fibrils of BC is formed by the bunching of microfibrils,

which are excreted from pores aligned on the surface of the cells in a row along their longitudinal axis. Cellulose nanofibers are synthesized from UDP-glucose by the Acetobacter cellulose synthase proteins AcsA and AcsB and secreted by AcsC and AcsD, forming an interconnected cellulose “pellicle” around cells [3, 21, 28, 31, 45].

4. Medical Applications

Bacterial cellulose (figure 4) has extensive application, in food industry, healthcare, cosmetics and in audio products [46]. Biomedical application include the use of BC for the production of contact lenses, electroconductive composite hydrogels biosensors, membranes for topical delivery of lidocaine and dietary supplements for combating diabetes, wound dressings, artificial skins and biomembranes [17, 47-50].



Figure 4. Images of bacterial cellulose (BC)[51].

MC membranes can also be infused with compounds that are known to promote healing, for example, superoxide dismutase (antioxidant), poviargol (antibiotic) [52]. Wan et al. [53] create a microbial cellulose membrane coated with hydroxyapatite (HAp), HAp-BC nanocomposites are important for applications as artificial bones and scaffolds for tissue engineering. Various others BC composites have been synthesized, BC-Silver nanoparticles for antimicrobial wound dressing and mask [54-56], BC-Paraffin for Bone scaffolding [57], Poly(vinyl alcohol)- bacterial cellulose for artificial dura mater (the membrane surrounding brain tissue) [58].

Bacterial cellulose is entirely utilize in the making of an artificial blood vessels (figure 5) for microsurgery and can be molded into tubular form. Klemm et al. [59] have designed a

matrix in order to produce a BC tube (diameter 1mm, length 5mm, wall thickness 0.7mm) with a tensile strength comparable to that of normal blood vessels (800mN). The tubes prepared by these authors were used to replace part of the carotid artery (4-6mm) of a rat. The BC implants were attached in an artificial defect of the carotid artery for one year. The BC-based blood vessels were shown to be stable vascular conduit and biocompatible[14,60]. Andrade et al.[61] have coated BC with the tripeptide Arg-Gly-Asp (RGD) to favor endothelialization and improve hemocompatibility of BC. They cultured human microvascular endothelial cells on RGD-coated BC. The results showed that the endothelial cells formed a confluent layer, inhibiting the adhesion of platelets.

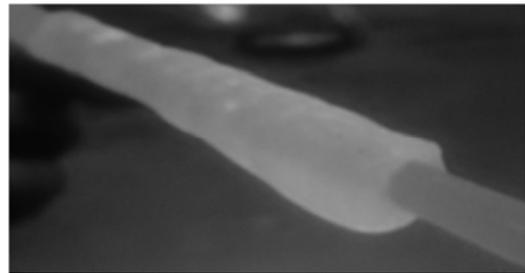


Figure 5. Bacterial cellulose tube that can used as blood vessel replacement [11].

The bacterial cellulose/gelatin composite scaffolds were prepared by Cai and Kim [62], with the incorporation of gelatin in the bacterial cellulose (figure 6), the crystallinity index tended to decrease while the thermal stability was improved. After the incorporation of gelatin in the bacterial cellulose, Young's modulus of the composite was increased from 3.7GPa to 3.9GPa, while the tensile strength and strain at break point were decreased from 170MPa (7.5%) to 114MPa (4%), respectively. Cell adhesion studies were carried out using 3T3 fibroblast cells. The cells incubated with BC/gelatin scaffolds for 48h were capable of forming cell adhesion and proliferation. It showed much better biocompatibility than pure bacterial cellulose. So, the prepared BC/gelatin scaffolds are bioactive and may be suitable for cell adhesion/attachment.

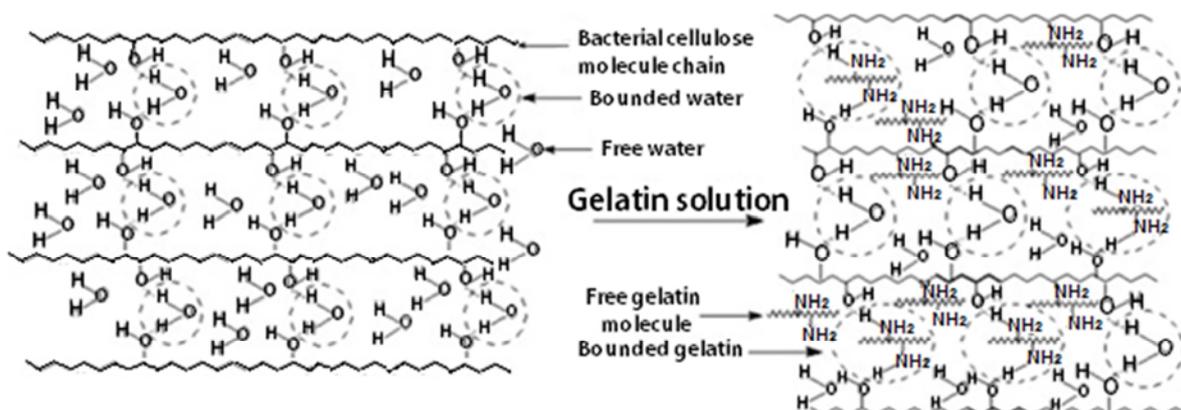


Figure 6. Bacterial cellulose/gelatin composite: the gelatin molecules penetrate between the individual nanofibers of the bacterial cellulose[62].

Yamanaka et al. [63] developed a process for the creation of long, hollow, microbial cellulose tubes with an i.d. of 2-6mm. These MC tubes could be used as replacement blood vessels or other tubular structures such as the ureter, the trachea, or the digestive tract. There are three basic requirements for the construction of an artificial vessel: (a) a sufficient structural scaffold which provides the desired shape and support for cell growth, (b) the proliferation of vascular cells, and (c) a proper nurturing environment [64]. The hollow microbial cellulose tubes proved to be biocompatible, especially with blood, and exhibited high durability. Tests on animal models revealed a slight adhesion of thrombi in the sutured portion, but no substantial adhesion of thrombi was observed on the inner surface of the blood vessel, leaving the center portion of the tube unobstructed [63].

Some investigators are using MC in the treatment of other tissues, for instance, Mello et al. [65] described an interesting application of MC in the field of modern neurosurgery, authors replace a portion of the brain's fibrous outer membrane with microbial cellulose. To improve the efficacy and safety of dural repair in neurosurgical procedures, a new dural material derived from bacterial cellulose was evaluated in a rabbit model with dural defects by Xu et al. [66]. Dura mater is a tough bilayer membrane tissue situated between the brain surface and the inner side of the skull. Its major function is to protect the brain and spinal cord. Artificial dura mater as a foreign body may cause inflammation. Authors showed that BC membranes evenly covered the surface of brain without adhesion. There were seldom inflammatory cells surrounding the membrane during the early postoperative period. The expression of inflammatory cytokines IL-1 β , IL-6 and TNF- α as well as iNOS and COX-2 were lower in the BC group compared to the control group at 7,14 and 21 days after implantation. Authors suggest that BC may become an ideal dural substitute material with vast potential neurosurgical applications because BC can repair dural defects in rabbit and has a decreased inflammatory response compared to traditional materials [66].

Microbial cellulose has been shown to be an highly effective wound dressing material. The results of various studies indicate that topical application of MC membranes improve the healing process of burns and chronic wounds. A recent study conducted in Poland used never-dried MC membranes in order to treat patients with severe second-degree burns. This study showed that the skin of the patients whose burns were covered with never-dried MC membranes healed faster (faster re-epithelialization) than the wounds of patients who received a conventional wound dressing, such as wet gauze and ointments [67]. Another example of microbial cellulose product, called Biofill, proved to be a very successful wound covering for skin problems such as burns and chronic ulcers. Biofill provide pain relief, protect the wound against infection, accelerate the healing process and reduce the cost of treatment [68-69]. In another study, Pornprom Muangman et al. [70] applied the microbial

cellulose, called Nanocell, to the face wound only once, without any further dressing change, progress of healing, until full epithelialization on the face, was observed for 2 weeks, during the treatment period, the patient did not show any irritation or allergic reaction to this new dressing, and wound swab culture showed no evidence of bacteria presence. These authors have observed that the use of microbial cellulose dressing in burn wounds provides a moist environment, a cooling effect, and activation of wound healing, as well as improvement in pain reduction.

5. Conclusion

Biocellulose (BC) is pure extracellular cellulose that is formed by many micro-organisms. BC received ample of attention due to its unique physiochemical properties. It can replace plant-based cellulose in multifarious applications. BC has proven to be a remarkably versatile biomaterial. In fact, biomedical devices recently have gained a significant attention owing to increase in tissue-engineering products for both wound care and the regeneration of demand or diseased organs. BC could function as a skin tissue repair material well, wound healing and regenerative medicine, guided tissue regeneration, periodontal treatments, or as a replacement for dura mater. BC is an interesting emerging biomaterial, with no toxicity, and since its discovery has shown tremendous potential as an effective biopolymer in various fields, because the structural aspect of BC is far superior to those of plant cellulose.

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