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Microbial Cellulose — Biosynthesis Mechanisms and Medical Applications

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Abstract

Currently some principles of sustainability, eco-efficiency and green chemistry are guiding the development of a new generation of materials as an alternative to conventional polymers based on petroleum. Then, in the field of biodegradable polymers one of the most promising investigations is focused on the use of microbial cellulose (MC), biocellulose or bacterial cellulose. MC has received substantial interest since it is synthesized from the bacterium *Gluconacetobacter genus* from a variety of carbon sources such as glucose, fructose, galactose, etc. MC is an interesting emerging biomaterial, with no toxicity, and since its discovery has shown tremendous potential in various fields, because the structural aspect of MC is far superior to those of plant cellulose. Thus, the main focus of the chapter review involves detailed aspects about the biosynthesis and recent advances on microbial production, including mechanism for the biochemistry of the cellulose synthesis, new sources for culture medium, main aspects about static and air-reactor productions and genetic modifications. We also revised microbial cellulose devices for biomedical applications: artificial skin, artificial blood vessels and microvessels, wound dressing of second- or third-degree burn ulcers, scaffolds for tissue engineering, drug delivery systems, dental implants, among others.

Keywords: Microbial cellulose, cellulose synthesis, medical applications

1. Introduction

Microbial cellulose (MC) presents the same chemical formula as plant cellulose, however with the fibers in nanometer dimensions; confer different properties to MC [1]. The MC is a type of exopolysaccharides composed of glucose monomers bound by glycosidic β (1-4) linkages, with

the chemical formula $(C_6H_{10}O_5)_n$, as can be seen in Figure 1. [2,3]. This biopolymer is produced extracellularly into nanofibers by several genera of bacteria, such as *Gluconacetobacter*, (formerly *Acetobacter*), *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Rhizobium*, *Sarcina*, and *Salmonella* [4].
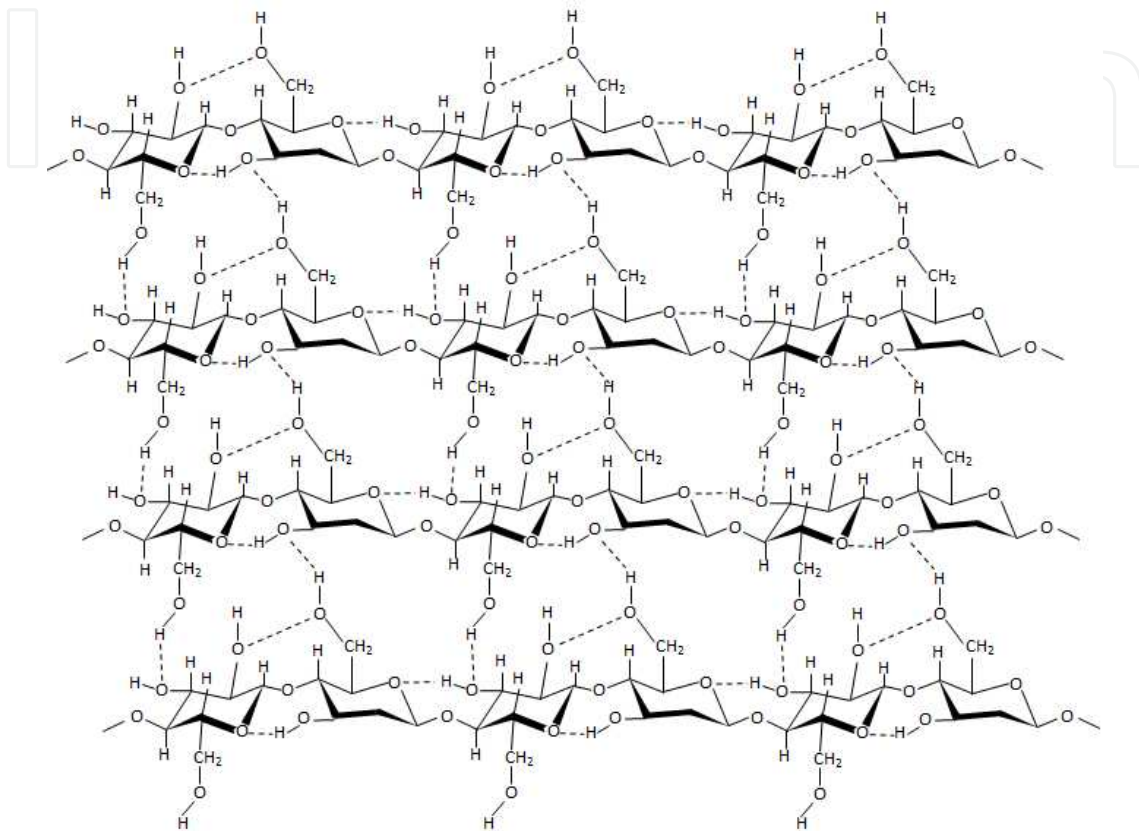

Figure 1. Chemical structure of microbial cellulose.

Historical data show that the MC has been used for a long time in the manufacture of a traditional food in the Philippines, known as the coconut cream [5]. Currently, the MC still remains widely utilized as food in various parts of the world however on the other hand aroused great academic and industrial interest due to its unique properties and diverse opportunity of applications. MC produced by *Acetobacter xylinum* was first reported in 1886 by Brown [6], produced in the presence of oxygen, using glucose as a carbon source.

As proposed by Yamada and colleagues (1997) [7] and subsequently validated by International Journal of Systematic Bacteriology, *Acetobacter xylinum* was reclassified and scientifically cataloged as *Gluconacetobacter xylinus*, due to the characteristics phylogeny based on analysis of partial sequences of 16S ribosomal RNA.

This bacterial species belonging to the family Acetobacteriaceae, being morphologically classified as a bacillus Gran-negative, strictly aerobic, no pathogenic which may be found singly arranged, in pairs or in small sets of chain formation of colonies shiny and smooth in

Mannitol Agar. Bacteria belonging to this family are able to oxidize fully various carbon sources such as glucose, fructose, galactose, sucrose, mannitol, glycerol, inositol, among others [3,8] and alcohols such as ethanol [7] and is capable of extracellularly producing pulp at temperatures between 25 and 30 °C and pH 3 to 7. The bacterial cellulose may provide arrangements in parallel via hydrogen bonds and to form a tridimensional network. The morphology of the membrane depends directly on the environment and the interface culture medium / air where MC form a thick film, which can be easily manipulated according to the size of the vial used for cultivation [1,8].

Researchers have sought new bacterial strains capable to produce biopolymers with potential industrial application [9]. Although many organisms are capable to produce cellulose, *Gluconacetobacter xylinus* bacteria are the only known species able to produce cellulose on the industrial scale [10].

In addition *G. xylinus*, other micro-organisms are considered able to produce cellulose, among others they are *Escherichia coli*, *Salmonella spp.* [11] and *Pseudomonas spp.* [12,13]. The cellulose synthesis genes (*MCsA, MCsB, MCsZ and MCsC*) of these species were similar to those in *G. xylinus* [14]. Although these species are also capable to produce the bacterial cellulose, the fact that many of them are potentially pathogenic limits the commercial use of these biopolymers [12,13].

In nature, microorganisms which produce cellulose are usually found in symbiosis with other microorganisms. In the fermentation of Kombucha, for example, *Zygosaccharomyces* yeasts are used as microorganisms symbionts [15]. The Kombucha, also known as tea fungi and *Haipao* [16], is produced in China for over 2000 years, with a widely varied yeast population [17,18], whose function is to convert sucrose to organic acids, carbon dioxide and ethanol, the latter being used for the cellulose-producing bacteria for the production of acetaldehyde and acetic acid [19,20].

2. Biochemical and molecular mechanisms of bacterial cellulose biosynthesis by *Gluconacetobacter xylinus*

Although several species of microorganisms are capable to produce cellulose, *G. xylinus* is currently considered a model organism for the study these biopolymers [21]. MC biosynthesis consists of a complex process which involves first the polymerization of glucose residues in β1-4-glucan chain [21,22,23], followed by the extracellular secretion of the chains ending the linear arrangement and crystallization of glucan chains through hydrogen bridges and Van der Waals forces hierarchically arranged in strips [22], resulting in formation of a tough three dimensional structure called microfibrils. MC generated by this bacterial species has special characteristics of unidirectional polarity and variable thickness. The crystallization mechanism of the microfibrils in *G. xylinum* can give rise to two cellulose forms, if the microfibrils is oriented parallel arrangement is synthesized cellulose I, while if the arrangement is antiparallel microfibrils is obtained cellulose II [21]. In *G. xylinus*, MC synthesis depends on the cycle of pentoses and of the Krebs cycle [1,21,22] that perform respectively the oxidation function

carbohydrates and oxidation of organic acids. A particularity *G. xylinus* is the inability to metabolize glucose anaerobically due to lack of phosphofructokinase-1, an enzyme responsible for catalyzing the reaction of phosphorylation of fructose-6-phosphate, fructose-1,6-bisphosphate, which prevents glycolysis. Thus, 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 [1,22]. 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. As previously mentioned, the cellulose synthesis can also occur from endogenous sources, for gluconeogenesis. In *G. xylinus*, the synthesis from endogenous sources begins with oxaloacetate, into pyruvate by action of the enzyme pyruvate carboxylase. The transformation of the pyruvate in fosfoenolpiruvado, is produced by action of the enzyme phosphoenolpyruvate carboxykinase [1,22,24]. MC synthesis reaction is costly to the cell, consuming about 10% of the ATP generated in bacterial metabolism. Thus, the energy used for the synthesis of CB comes from aerobic metabolism. There are different proposals for the substrate used by the CS. [1,21]. The enzymatic complex of synthesis of cellulose, termed as terminal complex (TC) [25,26], constitute a kind of membrane protein species that, in *Gluconacetobacter spp.*, corresponds to cellulose synthase complex [27].

One proposed hypothesis is that the UDP-glucose binds to lipids of the plasma membrane [1, 21]. Another one considers that the soluble precursor interacts directly with the CS [28]. The CS is a protein complex consisting of three (AxCcSAB, AxCcSC and AxCcSD) or four (AxCcSA, AxCcSB, AxCcSC and AxCcSD) protein subunits encoded by genes exist in an operon chromosomal called *MCs*.

The two conserved Asp residues (D) invariably are found in loops at the Carboxyl-terminal (C-terminal) ends of predicted strands, a position frequently observed for catalytic residues [21, 39]. The hydrophobic clusters in domain B are more difficult to interpret in terms of secondary structure. AxCcSA and AxCcSAB have a motif consisting of domain, a single conserved residues Asp (D-D-D), presumably important for catalysis, identified along with the conserved sequence motif Gln (Q) Arg-Trp (R-W) in glucotransferases [1, 29]. Through a functional analysis of CS, it appears that the A subunit of this complex with 83kDa, shows catalytic activity. The B subunit of 90kDa, increases the rate of cellulose synthesis by joining a positive allosteric regulator, cyclic diguanosine monophosphate (c-di-GMP). The C subunit (138kDa) and D (17kDa) appear to structural activity. It has been hypothesized that C subunit related to pore formation and extrusion of the cellulose D subunit appears related to the process decrystalization since mutant strains of the gene which encodes the D subunit production are still able to produce cellulose II [23].

In *G. xylinus* Bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) has been identified as an activator of cellulose biosynthesis [30], considered a second global messenger in bacteria [31]. The free c-di-GMP in the cell is considered to allosterically activate the cellulose synthase BcsA. However, 90% of the cellular c-di-GMP is reversibly bound by the c-di-GMP binding protein BcsB, a membrane protein that is structurally associated with the cellulose synthase [32,33]. It is believed that the spatial proximity is necessary to direct c-di-GMP released from BcsB towards the cellulose synthase. The equilibrium between bound and free c-di-GMP is modulated by the intracellular potassium concentration [34]. The level of free c-di-GMP is regulated by the opposing action of two enzymes, diguanylate cyclase (DGC) that cycles two molecules of GTP under the release of two molecules of PPi, and phosphodiesterase A (PDEA) that degrades c-di-GMP to the inactive GTP under the release of two molecules of PPi, and phosphodiesterase A (PDEA) that degrades c-di-GMP to the inactive 5'-pGpG. *G. xylinus* has three distinct operons each containing PDEA/DGC pair, which contribute at different levels to the c-di-GMP turnover [35, 36] indicating that cellulose biosynthesis underlies various control mechanisms in *G. xylinus*. Figure 2 represents a model of the metabolic pathway for the biosynthesis of cellulose by *G. xylinus*.

Figure 2. Hypothetical model of the pathway for the biosynthesis of cellulose by G. xylinus from exogenous sources - glucokinase-ATP (1); Phosphoglucomutase (2), glucose-6-phosphate dehydrogenase (3); Phosphoglucoisomerase (4); Fructokinase ATP (5), Aldolase (6); Triosephosphate isomerase (7); Glyceraldehyde 3-phosphate dehydrogenase (8); Phosphoglycerate mutase (9), enolase (10); Pyruvate kinase (11); Pyruvate biphosphate kinase (12), pyruvate dehydro-genase(13); 6-phosphogluconate dehydrogenase (14); Phosphorribulose epimeraase (15); Phosphorribulose isomerase (16); Transaketolase (17); Transaldolase (18); Fructokinase (19); Aldehyde dehydrogenase(20); Alcohol dehydrogen-ase(21).

3. Cellulose Synthase (CS)

Two bacterial cellulose synthase operons (*bcs*) [23] were identified in the analyzed genome, but only one (*bcs*I) is structurally complete. This operon is composed by seven genes encoding for enzymes endo-1,4-beta-glucanases, a homolog CMCax is a putative beta-glucosidase endoglucanase, CPC is a putative homologues four subunits CS: BCSA, BBRC, BCSC and BCSD and beta-glucosidase. Although the three endoglucanases have been identified [37] its exact function in MC biosynthesis is not well understood. Genomic Analysis showed that *bcs*D gene is conserved as part of the operon and has exactly the same length in all six strains. Interestingly, this gene encodes for CS subunit D, whose function is still speculative, although their crystal structure has been recently resolved.

In cells actively producing cellulose, approximately 50 cellulose-synthesizing multienzyme complexes are organized in a single row along the longitudinal axis of the bacterial rod whereby each complex secretes approx. 12 to 25 glucan chains which assemble into larger microfibrils at the site of synthesis. This so-called linear terminal complex can be visualized by electron microscopy using freeze fracture as 35 Å pores in the outer membrane or as pits when the outer leaflet is fractured away [1,33].

In *G. xylinus* specie the cellulose synthase complex (BCS) is a multicomponent protein complex encoded in an operon containing at least three genes, *bcs*A, *bcs*B, and *bcs*C, which encodes a transmembrane complex over the cytoplasmic and outer membrane whereby the cellulose synthase (BcsA) and the c-di-GMP binding protein (BcsB) are considered to be localized in the cytoplasmic membrane as shown in Figure 3. [32,33,39]. Cellulose synthesis and transport across the inner bacterial membrane is mediated by a complex of the membrane-integrated catalytic BcsA subunit (green) and the membrane-anchored, periplasmic BcsB domain (blue) and membrane-associated regions BcsB transmembrane anchor (blue). The glycosyltransferase domain is shown brown [40]. BcsC is predicted to form a β-barrel in the outer membrane, preceded by a large periplasmic domain containing tetratricopeptide repeats likely involved in complex assembly [41,42].

The gene corresponding to cellulose synthase BcsA is constituted by a long string that presents between 723-880 amino acid residues, as represented in Figure 3, being the most conserved gene of the operon *MCs* between species, although the amino-terminus portions (N-terminus) and carboxyl-terminus portions (C-terminus) is not so conserved, since the homology is not restricted to the frequently analyzed D, D, D35Q (R, Q) and RW motif, which spans domains A and B [39].

The BcsB protein [Figure 3], related to indirect interaction with c-di-GMP [33] is less well conserved among the species. However, direct comparisons of the MCsB proteins with CelB from *A. tumefaciens* and *R. leguminosarum* bv. *trifolii* revealed significant homology (~40% similarity) over the entire length of the proteins with several invariable residues. An alanine/proline rich domain is located at the N-terminus region of all proteins except *A. aeolicus*. One transmembrane domain located at the C-terminus portions has been predicted by various algorithms for all BcsB proteins [39].
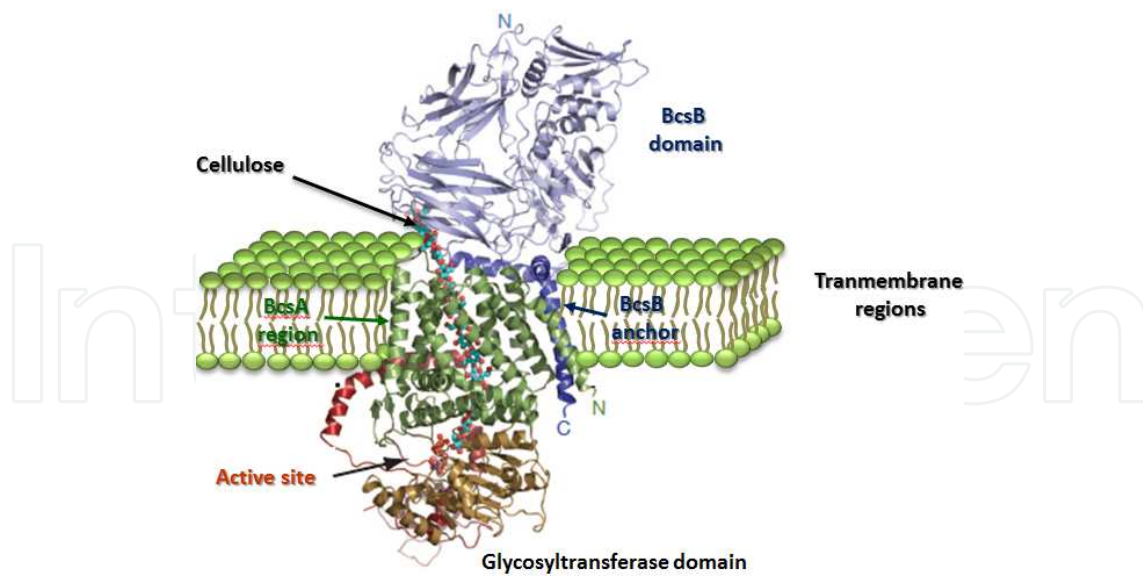
Figure 3. Schematic representation of cellulose synthase.

The proposed model considers that after the transfer and addition of glycosyl terminal residue, the glucose molecule rotates around acetyl glucan binding to align the channel as shown Figure 4. It is believed that allosteric interactions guide the direction of rotation, causing rotation feature 180⁰ connecting β-1,4 glucan-glucan between the individual glucose units and intramolecular hydrogen bond between oxygen atoms of the hydroxyl groups of the neighboring unit [40,43]. This phenomenon may be sufficient to allow the polymer to move into the channel [Figure 4]. Alternatively, for the translocation of the elongated glucan occurs, replacement is required to UDP-glucose by UDP. Past the channel, induced glucan chain in the BcsA - twist BcssB interface, interaction with BcsB of CBDs (periplasmic carbohydrate binding domains), or aggregation with other glucans may additionally contribute to a unidirectional motion of the polymer [40].
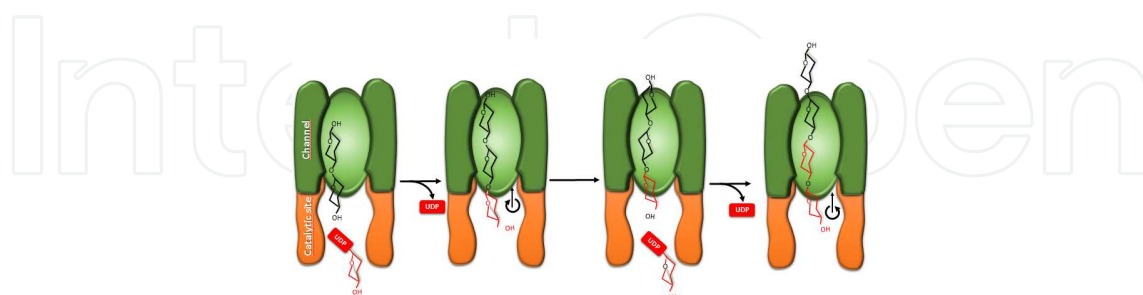
Figure 4. Proposed model for cellulose synthesis and translocation (adapted). After glycosyl transfer, the newly added Glc could rotate around the acetyl linkage into the plane of the polymer. The rotation direction would be determined by steric interactions and formation of the β-1,4 glucan characteristic intramolecular O_3- H••O_5 hydrogen bond. The glucan might translocate into the channel during this relaxation. This process would be repeated with a second UDP-Glc but the rotation direction after glycosyl transfer would be in the opposite direction owing to steric constraints. Alternatively, the glucan might not translocate into the channel until UDP is replaced by UDP-Glc. Trp 383 and Cys 318 mark the entrance to the transmembrane channel [40].

4. Cultivation conditions for production of bacterial cellulose

The MC production depends of the appropriate cultivation conditions, which include the composition of the culture medium (synthetic and natural media), temperature, pH and methods agitated or static cultivation. The choice of condition cultivation or another depend on the purpose, once these conditions have significant influence on the properties of structure, physical and mechanical MC.

The current methods of MC production are static culture [44], submerged fermentation through aerated or agitated cultivation [45], and the airlift bioreactor [46]. Large scale, semi-continuous and continuous fermentation are dominant to meet commercial demand. In all cases, the main objective is to achieve maximum production of MC with optimum form and suitable properties for the application for which it is intended. After all, a wider application of this versatile biopolymer depends on the practical considerations such as the scale-up capability and production costs. *G. xylinus* has two main operative amphibolic pathways: the pentose phosphate cycle for the oxidation of carbohydrates and the Krebs cycle for the oxidation of organic acids and related compounds [12,21,47,48]. Consequently, several studies have been reported that the composition of the culture medium and the fermentation conditions significantly affect the order structure of cellulose [49,50,51].

Static cultivation is a relatively simple and widely used method of cellulose production. The medium is placed into shallow tray or bottles, inoculated, and cultivated for several days until the cellulose nearly fills the tray. *G. xylinus* produces a gelatinous MC membrane, which has a denser surface on the side exposed to air, i.e. capable of generating cellulose as an extracellular product on static media (at the air-medium interface) at temperatures between 25 and 30 °C and pH from 4 to 7 [52]. The traditional static culture represents an expensive way of MC production that may hinder its industrial application since the productivity is low and long cultivation time is required. Consequently, authors have proposed new culture system as strategy to increase the MC productivity to a suitable for commercial applications in simple fed-batch [53], in bioreactor for a semi-continuous production [54], in a modified airlift-type bubble column bioreactor [55].

Nutrients required for the growth of these microorganisms are carbon and nitrogen sources, phosphorus, Sulphur, potassium and magnesium salts [56]. Sometimes a complex medium supplying amino acids and vitamins is also used to enhance the cell growth and production [57]. Between the years 1940 to 1960, researchers at the Hebrew University in Jerusalem intensively investigated the biochemistry of simplified production and quantification of cellulose cellulose for produced by *G. xylinus*. These media, named as HS are widely used nowadays [44]. Typical carbon sources in the production of microbial cellulose include glucose, fructose, sucrose, mannitol, however among others including arabinose, arabitol, citric acid, ethanol, ethylene glycol, diethylene glycol, galactose, glucono lactone, glycerol, inositol, lactose, malic acid, maltose, mannose, methanol, rhamnose, ribose, sorbose, starch, succharide, succinic acid, trehalose, and xylose have been also investigated [58,59,60] to maximize bacterial cellulose production by various *Gluconacetobacter* strains [8]. The best yield was obtained in fed-batch fermentation, 15.3 g/L in 50 hours of cultivation using glucose as carbon source [61]

and other examples of different culture conditions can be found in Table 1. The results shown in Table 1 indicate that, glucose seems to be the best carbon source. It demonstrates the validity of the results that various carbon substrates could be converted to monomer glucose by *Gluconacetobacter*, followed by polymerization to MC [60]. Among the factors affecting cost, carbon sources play a major role in fermentation [47,62]. Conversion of 60–80% of the utilized carbon source into crude polymer is commonly found in high yielding polysaccharide fermentations. In order to compensate its low sugar conversion yield and to reduce the feedstock cost of MC production, in recent years, MC has been produced by fermenting the hydrolysates of agricultural wastes such as hemicelluloses [63], konjac powder [62], rice bark [64] and waste cotton fabrics [63,65]. An advantage of using agricultural or industrial residual streams as feedstock is the low or no value of the raw material. Several successful efforts have been made to use certain industrial food wastes as growth medium for the MC producer organisms, which is not only a cheap way but also works as a basin for environmental cleaning [51]. Thin stillage (TS) is a wastewater from rice wine distillery rich in carbon sources and organic acids. [66] discovered that TS, when employ to replace distilled water for preparing Hestrin and Schramm medium (the traditional MC production medium), can enhance the MC production 2.5-fold to a concentration of 10.38 g/L with a sugar-MC conversion yield of 57 % (0.57 g MC/g reducing sugar) after 7 days of static cultivation. In 2012, Ha et al. [48] further improved the MC production, 15.28 g/L of MC was obtained after 15 days of cultivation. Yeast extract and peptone are the most commonly used nitrogen sources in MC production as they provide nitrogen and growth factors for *Gluconacetobacter* strains. Many researchers are trying to find efficient substitutes due to their high cost. Even if various nitrogen sources were added to the HS medium, peptone is found to be the most effective nutrient. However, corn steep liquor (CSL) which produced the second highest production is always chosen as a substitution for the economic viewpoints. Buffering capacity is also important for MC production. Insoluble MC often attaches to pH probe and leads to inaccurate reading [4]. Noro et al. [67] pointed out the buffering capacity of CSL, which could maintain the pH within the optimal range during the production of MC. Jung et al. [68] doubled MC production (from 1.53 to 3.12 g/L) using molasses as carbon source and corn steep liquor as nitrogen source when compared with the results obtained from complex medium. This strategy could not only reduce burden on environment but also achieve the goal of large scale production with low cost. The optimal pH for MC production may vary with carbon source. *G. xylinus* accumulates gluconic acid at low pH, and a preferred environment for both biomass and MC production can be achieved by shifting pH from 4.0 to 5.5 during cellulose production phase in fed-batch cultures [61]. Under static batch cultivation, the pH of the culture medium decreases due to the respiratory metabolism of *G. xylinus*, which involves the ethanol oxidation to acetic acid and the glucose conversion into gluconic acid. This fact makes it very important to control the pH within the optimum range for cell growth and cellulose production [48,69]. *Gluconacetobacter* strains require oxygen as an essential substrate, consequently volumetric oxygen transfer coefficient (kLa) is a key limiting factor in the aerobic fermentation for producing MC. Song et al. [55] investigated the optimum aeration rate for a 50-L spherical type bubble column bioreactor, and it was determined to be 1.0 vvm (30 L/min).

Attempts to enhance MC production by adding different additives in the fermentation medium have been made. The possible mechanisms of these various additives to enhance MC were also proposed such as reduction of the shear force by increasing the viscosity of medium [70]. Different chemical compounds including alcohols [72], glycerol [60,68], organic acids [68], polysaccharides [72] thin stillage from rice wine distillery [66] and thin stillage from beer culture broth [47,53] have been used as additives to the fermentation medium with the aim of increasing MC production.

Microorganism	Carbon source	Cultivation mode	Time culture (days)	MC production (g l⁻¹)	Reference
G. xylinus (BRC 5)	Glucose	Fed-batch	2	15.3	Hwang *et al.* (1999) [61]
G. xylinus (BPR 2001)	Fructose	Agitated	3	14.1	Bae *et al.* (2004) [70]
G. xylinus (KJ1)	Saccharified food wastes	airlift-type bioreactor	3	5.6	Song et al. (2009)[55]
A. xylinum (ATCC 700178)	CSL-Fru	Agitated	5	13.0	Cheng *et al.* (2011) [72]
Gluconacetobacter sp (F6)	Glucose	Static	6	4.5	Jahan *et al.* (2012) [73]
G. xylinus (MCRC 12334)	TS-Glu	Static	7	10.38	Wu *et al.* (2012)[66]
G. xylinus and Trichoderma ressei	Glucose	Static	14	6.23	Cavka *et al.* (2013) [74]
G. xylinus (PTCC, 1734)	Syrup	Static	14	43.5	Moosavi-Nasab and Yousefi (2011) [75]
G. xylinus (ATCC 23769)	Glucose	Static	15	15.28	Ha and Park (2012) [76]

Table 1. Bacterial cellulose production under different culture conditions

5. Microbial cellulose for biomedical applications

As previously described, intense research has focused on the use of natural biopolymers in a variety of biomedical materials and devices, including wound dressings, medical implants, drug delivery, vascular grafts, and scaffolds for tissue engineering [78]. Consequently,

continual efforts from many researchers, led to novel systems that closely mimic the complex and hierarchical structures inherent to the native tissue are sure to emerge.

In the last decade, several nanocellulose-based materials have been created for a diversity of biomedical applications. Some review articles have highlighted the potential applications of cellulose materials [72,79,80,81,82].

MC represents an interesting emerging nanomaterial, with no toxicity, and since its discovery has shown tremendous potential as an effective biopolymer which offers a wide range of applications, especially the biomedical ones, including the use as biomaterial for artificial skin, artificial blood vessels and microvessels, wound dressing of second- or third-degree burn ulcers and dental implants. Other studies with endothelial, smooth muscle cells and chondro-cytes have shown that these cells present good adhesion to bacterial cellulose. [83]

5.1. Pristine MC based biomaterials

One of the main direct applications of MC membranes in biomedical field is related to wound dressing. Fontana et al. [84] were the pioneers in describing the use of bacterial cellulose to replace burned skin. Since then, literature shows a great number of papers related to wound dressing. Cellulose dressings are recommended as a temporary covering for the treatment of wounds, including pressure sores, skin tears, venous stasis, ischemic and diabetic wounds, second-degree burns, skin graft donor sites, traumatic abrasions and lacerations, and biopsy sites by the manufacturers [85].

MC based wound dressings are commonly available on the market nowadays, for example: BioFill®, Bioprocess®, XCell® and Gengiflex® (for periodontal diseases reconstruction [86]. The biomembrane BioFill® was one of the first commercial product that fulfills the main prerequisites of an ideal wound dressing, including: low cost, good adherence to the wound, water vapor permeability, elasticity, transparency, durability, it constitutes a physical barrier for bacteria, is hemostatic, it presents easy handling and application with minimum exchanges. BioFill® effectiveness has been proven in more than 300 cases in accelerating the healing process, pain relief, etc. [86,87,88,89].

Despite the analgesic mechanism of action of these dressings has not been fully elucidated, some authors suggest that the healing mechanism involves the capture of ions by means of cellulose hydrogen bonds, or the nano MC 3-D network mimics the skin surface creating optimal conditions for healing or regeneration [87,88,89].

It is important to point out that MC wound dressing clearly shortened the time to heal or wound closure over standard care when applied to non-healing lower extremity ulcers, as observed by many researchers [88,89,90]. As can be seen in Figure 5, novel applications of wet MC as wound dressing in the treatment partial thickness burns were applied by Czaja et al. [88,89] presenting excellent results suggesting that MC as a wound dressing promotes a favorable moist environment for a fast wound cleansing, and consequently for rapid healing.

Figure 5. Bacterial cellulose dressing applied on wounded torso and face. (Reprinted with permission from Czaja, W. K. et al. (2007). The future prospects of microbial cellulose in biomedical applications, Biomacromolecules, Vol.8, No.1, pp. 4. Copyright (2007) American Chemical Society).

Other interesting biomedical applications for MC films have emerged. A Brazilian research group had designed and patented a device to manufacture MC contact lens for therapy in cases of regeneration of cornea [91].

In terms of vascular applications, a German group created BASYC® (Bacterial Cellulose Synthetised), consisting of a tubular biomaterial for applications in microsurgery of arteries and veias [92] and [93] applied vascular stents in animals arteries. Bodin et al. [94] obtained MC tubes by modifying the fermentation process of *Acetobacter xylinum* on top of silicon tubes, as shown in Figure 6.
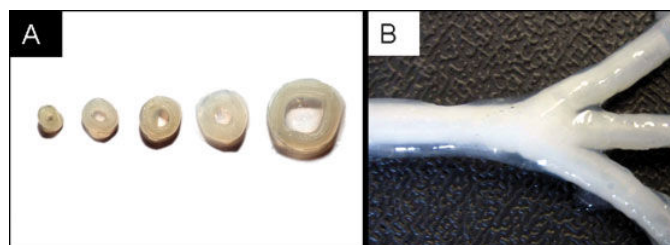
Figure 6. MC tubes presenting different sizes and shapes for applications: a) Mc tubes showing different inner diameters: 1.5mm, 2.4mm, 3.0mm, 4.0 mm and 6.0 mm. b) Branched MC tube fermented on a branched silicone tube. (Reprinted with permission from Bodin et al., 2007 Biotechnology and Bioengineering, Vol. 97, No. 2, June 1, 2007 [94])

Lately, Nimeskern et al. [95] designed and fabricated an ear-shaped pristine MC prototype material applying a Magnetic Resonance Imaging (MRI) scanning technique. This study was extremely important to confirm that MC is a promising tissue engineering material with appropriate mechanical properties for ear cartilage replacement. Thereby, it may be used to create patient-specific ear shapes.

5.2. Microbial cellulose nanocomposites for biomedical applications

Beyond the direct uses, MC can be widely and effectively utilized as either functional reinforcements or excellent matrices due to excellent mechanical properties and biocompatibility which

allows it to be engineered in various forms from nano to macro scale. Thus, MC based nanocomposites can be manipulated to improve their properties and/or functionalities becoming one of the reasons that makes MC so exceptional material for biomedical applications.

In relation to biomaterial applications for wound dressing and skin tissue repair several MC based nanobiocomposites were fabricated. Here are some examples: membranes of MC/ collagen [96], MC/gelatin [97], MC/ aloe vera films [98], MC/alginate for temporary dressing material [99].

Further, MC composite with kaolin was proved as short-term and long term wound healing materials [100].

Freeze-drying techniques allowed the preparation MC/poly(ethylene glycol) (PEG) composites of by immersing wet MC pellicle in PEG aqueous solution [101]. This same technique was applied by some of us [102] to obtain MC/ silk fibroin (SF) sponge scaffolds. *In vitro* tests proved non-cytotoxic or genotoxic character of these nanobiocomposites. SEM images revealed a greater number of fibroblast cells (L929 cell line) attached at the MC/SF:50% scaffold surface if compare with the surface of pure MC, suggesting that the presence of fibroin improved cell attachment as is possible to see in Figure 7. This could be related to the SF amino acid sequence that act as cell receptors facilitating cell adhesion and growth. Consequently, MC/SF:50% scaffolds configured an excellent option in bioengineering depicting its potential for tissue regeneration and cultivation of cells on nanobiocomposites.
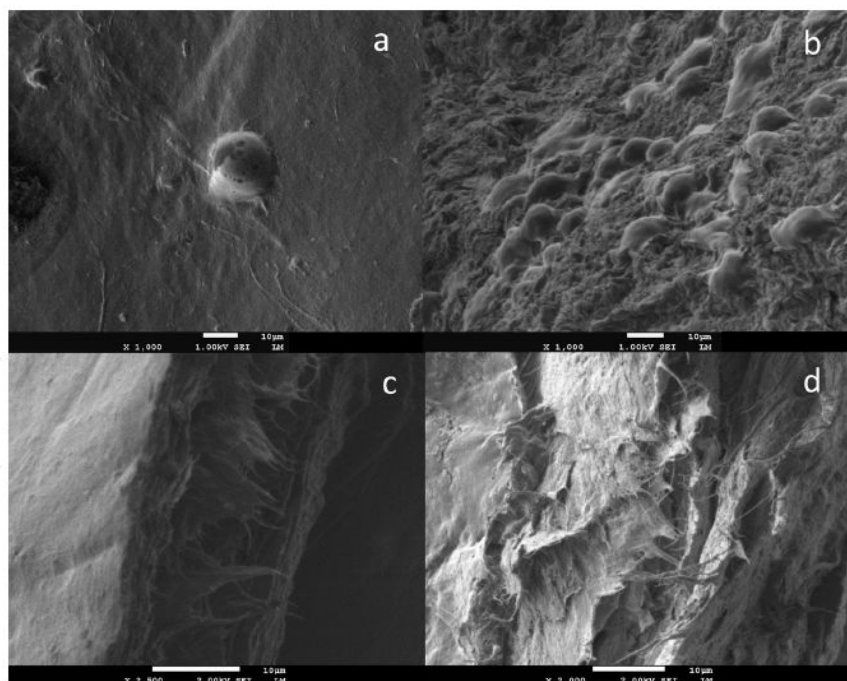
Figure 7. To test the hypothesis that the addition of silk fibroin to cellulose scaffolds increases cell adhesion (48 h), L-929 cells were seeded in MC and MC/SF scaffolds. SEM images of the cells attached to MC (a) and MC/SF (b) scaffolds surface; cross-section SEM images of MC (c) and MC/SF (d) evidenced that the cells did not migrate into the scaffolds. (Reprinted with permission from Oliveira Barud et al., 2015, Carbohydrate Polymers, Vol 128, April, 2015 [102]).

Generally, a scaffold provides a foundation for cell attachment, and several materials have been tested as scaffolds to support growth of cells. The need for bio-mimicking scaffolds has led to the exploration of MC as a scaffold material. There is an increased interest in developing adipose tissue as an *in vitro* model for adipose biology and metabolic disease, and to this end, 2D and 3D porous scaffolds of bacterial nanocellulose and alginate were prepared recently [103].

Bäckdahl et al. [104] also developed MC scaffolds with controlled microporosity by placing paraffin wax and starch particles during culture and removing these particles once the cultivation process was finished. The MC scaffolds were then seeded with smooth muscle cells for investigating the potential tissue engineered blood vessel application.

A variety of surface functionalization through biosynthetic or chemical modification was also investigated. Various approaches to the preparation of functional MC-based nanocomposites by incorporating different guest substrates including small molecules, inorganic nanoparticles or nanowires, and polymers on the surfaces of MC nanofibers are exemplified which can improve the functionality of MC nanomaterials and expand its potential application in the biomedical fields.

Nanocomposites were obtained by the association of nanoparticles presenting antimicrobial activities, including silver nanoparticles [105,106,107,108,109,110]. Additionally, Barud et al. [111] also prepared MC/propol membranes that presented good antimicrobial activities to be used as wound dressing material.

In recent years, several controlled release systems based on nanocellulose material for various pharmaceutical applications have been also investigated to delivery Tetracycline [112], benzalkonium chloride [113], topical release of lidocaine [114] and release of proteins with serum albumin [115].

With respect to bone regeneration in defects of rat tibiae, MC-hydroxyapatite (MCHA) nanobiocomposite were prepared to evaluate the biological properties and performance of the material [116]. The MCHA membranes were effective for bone regeneration and accelerated new bone formation. In addition, reabsorption of the membranes was slow, suggesting that this composite takes time to be completely reabsorbed.

6. Conclusion

Microbial cellulose is a natural renewable polymer synthesized from the bacterium *Glucona-cetobacter xylinus* that is the only known species capable to produce cellulose on an industrial scale. In an appropriate culture medium the bacteria secretes about 50-80 cellulose microfibrils from 3.0 to 3.5 mm thick, free of lignin and hemicellulose, which aggregate themselves to form strips arranged in a 3-D hierarchical network. Besides that MC configures one of the most promising investigations in the field of biodegradable polymers. Due to this uniform structure and morphology MC is endowed with unique characteristics such as high purity, high crystallinity and remarkable mechanical properties, good chemical stability, high water

holding capacity featuring it as a completely biocompatible polymer. Despite its high water content, MC shows a good mechanical performance and it can be produced in almost any shape due to its high moldability during formation. MC 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 MC is far superior to those of plant cellulose. Thus, this chapter reviewed involved detailed aspects about the biosynthesis and recent advances on microbial production, including mechanism for the biochemistry of the cellulose synthesis, new sources for culture medium, main aspects about static and air-reactor productions and genetic modifications. We also revised and presented a great number of different MC based materials that were designed for biomedical applications (dressings, scaffolds, drug delivery systems), among others. Additionally, we hope that this book chapter may aggregate high quality information and may be a benchmark to intensify greater interest of the scientific community in microbial cellulose and related devices and also inspire the development of new materials in this field.

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