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Properties and Applications of Bacterial Cellulose as a Biological Non-woven Fabric

A. ASHJARAN

Department of Chemistry and Textile, Shahre-rey Branch, Islamic Azad University, Tehran, Iran

Corresponding author: Tel: +98 228 2365536; E-mail: a.ashjaran@gmail.com

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The role of microbial technologies in utilization of the new raw material resources for fabrication of fibers, nonwoven fabric and films, in improving production of existing types of fibers and in creating new types of fiber materials of natural origin was evaluated. The potential capacity of microorganisms to form fiber structures, fiber and film forming polymers and the monomers for their chemical synthesis was analyzed. Nanofibers and biononwoven fabric of pure cellulose can be made from some bacteria such as *Acetobacter xylinum*. Bacterial cellulose fibers are pure, tens of nm across and about 0.5 μ long. The fibers are very stiff and, although nobody seems to have measured the strength of individual fibers. Their stiffness up to 70 GPa. Fundamental strengths should be at least greater than those of the best commercial polymers, but best bulk strength seems to about the same as that of steel. They can potentially be produced in industrial quantities at lowered cost and water content and with triple the yield, by a new process. This article presents a critical review of the available information on the bacterial cellulose as a biological nonwoven fabric with special emphasis on its fermentative production and applications. Characteristics of bacterial cellulose biofabric with respect to its structure and physicochemical properties are discussed. Current and potential applications of bacterial cellulose in textile, nonwoven cloth, paper, films synthetic fiber coating, food, pharmaceutical and other industries are also presented.

Key Words: Bacterial cellulose, *Acetobacter xylinum*, Nanofiber, Biononwoven, Biosynthesis.

INTRODUCTION

Polysaccharides can be divided according to their morphological localization as: intracellular polysaccharides located inside or as part of the cytoplasm membrane; cell-wall polysaccharides forming a structural part of the cell wall and extracellular polysaccharides located outside the cell wall[1-5]. Extracellular polysaccharides occur in two forms: loose slime, which is non-adherent to the cell and imparts a sticky consistency to bacterial growth on a solid medium or an increased viscosity in a liquid medium and microcapsules or capsules, which adhere to the cell wall. They have a definite form and boundary, being only slowly extracted in the water or salt solutions. It is, therefore, possible to separate capsules and microcapsules from loose slime by centrifugation[6-12].

Exopolysaccharides are long chain polysaccharides consisting of branched, repeating units of sugars or sugar derivatives, mainly glucose, galactose and rhamnose in different ratios. They are classified into two groups: homopolysaccharides (cellulose, dextran, mutan, pullulan,curdlan) and heteropolysaccharides (gellan, xanthan)[13,14]. Homopolysaccharides consist of repeating units of only one type of monosaccharides (D-glucose or D-fructose) joined by either a single linkage type (*e.g.*, 1 → 42 or 1 → 44) or by a combination of a limited number of linkage types (*e.g.*, 1 → 42 and 1 → 44). Heteropolysaccharides consist of multiple copies of oligosaccharides, containing three to eight residues, produced by a variety of microorganisms. Exopolysaccharides find wide industrial applications in food, pharmaceutical and other industries like textile, paper, cosmetics, gelling agents and medicines for wound dressing[15,16].

There are four principle sources of cellulose. The majority of cellulose is isolated from plants. A second source is the biosynthesis of cellulose by different microorganisms, including bacteria (glucon *Acetobacter xylinus*), algae and fungi among others[17-19]. The other two less common sources include the enzymatic *in vitro* synthesis starting from cellobiosyl fluoride and the chemosynthesis from glucose by ring-opening polymerization of benzylated and pivaloylated derivatives[20-23]. Bacterial cellulose (BC) is produced by strains of the bacterium glucon *Acetobacte xylinus*, which is a gram-negative, rod shaped and strictly aerobic bacterium. It has very high purity and contains no lignin, hemicelluloses, pectin and waxes as plant cellulose does. Bacterial cellulose differs from plant cellulose with respect to its high crystallinity, ultrafine network structure, high water absorption capacity, high mechanical

strength in the wet state and availability in an initial wet state and biocompatibility[22,24-26].

Intensive studies on bacterial cellulose synthesis, using *A. xylinum* as a model bacterium, were started by Hestrin *et al.*[39,43], who proved that resting and lyophilized Acetobacter cells synthesized cellulose in the presence of glucose and oxygen. Next, Colvin (1957) detected cellulose synthesis in samples containing cell-free extract of *A. xylinum*, glucose and ATP. Further milestones in studies on BC synthesis, presented in this review, contributed to the elucidation of mechanisms governing not only the biogenesis of the bacterial polymer, but also that of plants, thus leading to the understanding of one of the most important processes in nature. *Acetobacter xylinum* produces two forms of cellulose: (i) cellulose I, the ribbon-like polymer and (ii) cellulose II, the thermodynamically more stable amorphous polymer[27-31]. Nanofibrillar structure of bacterial cellulose is responsible for most of its properties such as high tensile strength, higher degree of polymerization and crystallinity index. Bacterial cellulose is used as a diet food and to produce new materials for high performance speaker diaphragms, medical pads[32,33] and artificial skin[22,34,35]. Relatively high cost of the production of cellulose may limit its application to high value-added products as well as speciality chemicals[28,32]. Significant cost reductions are possible with improvements in fermentation efficiency and economics of scale, the lower limit of the cost of microbial cellulose being determined by the price of the raw material substrates. Consequently, Acetobacter cellulose may always be more expensive to produce than conventional sources of cellulose[36,37]. For this reason, successful commercialization of Acetobacter cellulose will depend on careful selection of applications where its superior performance can justify its higher cost[34].

The molecular formula of bacterial cellulose $(C_6H_{10}O_5)_n$ (Fig. 1) is the same as that of plant cellulose, but their physical and chemical features are different[38,39]. Various strains producing cellulose are depicted systematically in Table-1.

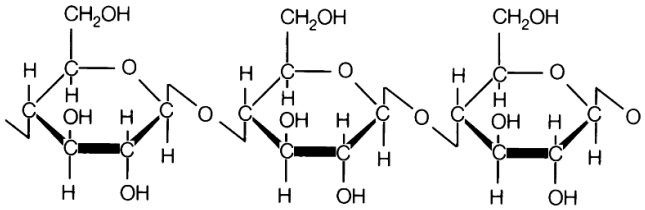

Fig. 1. Repeating units of cellulose[40]

Bacterial cellulose is preferred over the plant cellulose as it can be obtained in higher purity and exhibits a higher degree of polymerization and crystallinity index. It also has higher tensile strength and water holding capacity than that of plant cellulose, making it more suitable raw material for producing high fidelity acoustic speakers, high quality paper and dessert foods[36,41]. Fibrils of bacterial cellulose are *ca.* 100 times thinner than that of plant cellulose, making it a highly porous material, which allows transfer of antibiotics or other medicines into the wound while at the same time serving as an efficient physical barrier against any external infection. It is therefore used extensively in wound healing[23]. Microbial cellulose exists

TABLE-1
DIFFERENT STRAIN PRODUCING MICROBIAL CELLULOSE[40]

Microorganism	Carbon source	Supplement
A. xylinum BRC 5	Glucose	Ethanol, oxygen
G. hansenii PJK (KCTC 10505 BP)	Glucose	Oxygen
G. hansenii PJK (KCTC 10505 BP)	Glucose	Ethanol
Acetobacter sp. V6	Glucose	Ethanol
Acetobacter sp. A9	Glucose	Ethanol
A. xylinum BPR2001	Molasses	None
A. xylinum BPR2001	Fructose	Agar oxygen
A. xylinum BPR2001	Fructose	Agar
Acetobacter xylinum ssp. *Sucrofermentans* BPR2001)	Fructose	Oxygen
Acetobacter xylinum ssp. *Sucrofermentans* BPR2001)	Fructose	Agar oxygen
Acetobacter xylinum E25	Glucose	No
G. xylinus strain (K3)	Mannitol	Green tea
Gluconacetobacter xylinus IFO 13773	Glucose	Lignosulphonate
Acetobacter xylinum NUST4.1	Glucose	Sodium alginate
Gluconacetobacter xylinus IFO 13773	Sugar cane molasses	No
Gluconactobacter sp. RKY5	Glycerol	No
Co-culture of *Gluconacetobacter* sp. St-60-12 and *Lactobacillus mali* JCM1116	Sucrose	No

as basic structure known as microfibrils, which are composed of glucan chains interlocked by hydrogen bonds so that a crystalline domain is produced. This nanofibrillar structure of bacterial cellulose was first described by Mühlethaler[42]. Electron microscopic observations showed that the cellulose produced by *Acetobacter xylinum* occurs in the form of fibers. The bacteria first secreted a structurally homogeneous slimy substance within which, after a short time, the cellulose fibers were formed. Microbial cellulose as a bio nonwoven fabric can be used for fabrication of paper, special acoustic membranes, films, nonwoven cloth and synthetic fiber coatings[43-45].

Biocompatible biofabric from bacterial cellulose: All genes responsible for biocellulose synthesis have been cloned and their characterization is under way[46]. Fig. 2 shows the predicted steps of bacterial cellulose synthesis when glucose is used as the carbon source. The analysis of genes will lead to higher productivity of bacterial cellulose and to new biocellulose with different properties[24,47].

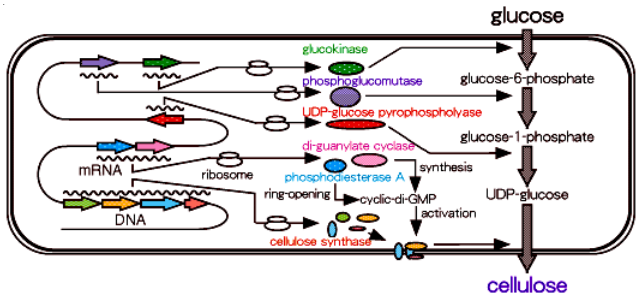

Fig. 2. Biosynthesis of bacterial cellulose from glucon acetobactor xylinum[46]

A. xylinum is a simple gram-negative bacterium which has an ability to synthesize a large quantity of high-quality cellulose organized as twisting ribbons of microfibrillar

bundles[48,49]. During the process of actual biosynthesis, various carbon compounds of the nutrition medium are utilized by the bacteria, then polymerized into single, linear b-1,4-glucan chains and finally secreted outside the cells through a linear row of pores located on their outer membrane. The subsequent assembly of the b-1,4-glucan chains outside of the cell is a precise, hierarchical process. Initially, they form subfibrils (consisting of 10-15 nascent b-1,4-glucanchains), then later microfibrils and finally bundles of microfibrils consisting of a loosely wound ribbon, which is comprised of about 1000 individual glucan chains[47,50]. The thick, gelatinous membrane formed in static culture conditions as a result of these processes is characterized by a 3-D structure consisting of an ultrafine network of cellulose nanofibres (3-8 nm) which are highly uniaxially oriented[51,52]. Such a 3-D structure, not found in vascular plant cellulose, results in high cellulose crystallinity (60-80 %) and an enormous mechanical strength. Particularly impressive is the fact that the size of microbial cellulose fibrils is about 100 times smaller than that of plant cellulose. This unique nanomorphology results in a large surface area that can hold a large amount of water (up to 200 times of its dry mass) and at the same time displays great elasticity, high wet strength and conformability. The small size of microbial cellulose fibrils seems to be a key factor that determines its remarkable performances a wound healing system. Furthermore, the never dried cellulose membrane is a highly nanoporous material that allows for the potential transfer of antibiotics or other medicines into the wound, while at the same time serving as an efficient physical barrier against any external infection. The cellulose produced in the form of a gelatinous membrane can be molded into any shape and size during its synthesis, depending on the fermentation technique and conditions used[53,54]. Unlike celluloses of plant origin, microbial cellulose is entirely free of lignin and hemicelluloses. A vigorous treatment with strong bases at high temperatures allows the removal of cells embedded in the cellulose net and it is possible to achieve a non-pyrogenic, non-toxic and fully biocompatible biomaterial (Fig. 3)[23,33,35,55].

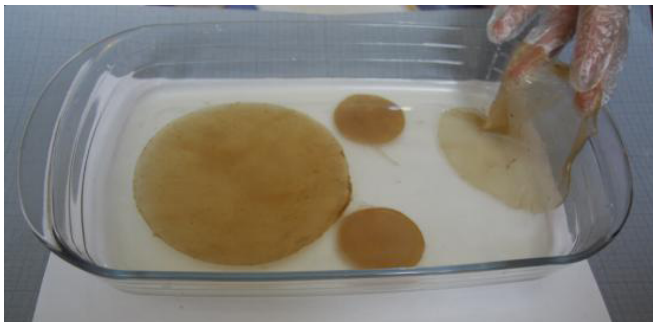

Fig. 3. Never-dried and fully biocompatible biofabric from bacterial cellulose[56]

Effect of medium on production of biofabric from bacterial cellulose: The fermentation medium contains carbon, nitrogen and other macro- and micronutrients required for the growth of organism. The changes in the medium components affect the growth and the product formation directly or indirectly. Secretion of exopolysaccharides is usually most noticeable when the bacteria are supplied with an abundant carbon source and minimal nitrogen source[40,57-59]. Sometimes a complex medium supplying amino acids and vitamins is also used to enhance the cell growth and production[60,61].

Last treatment of biofabric from bacterial cellulose: The microbial cellulose obtained after fermentation is not pure; it contains some impurities like cells and/or the medium components. Care must be taken in the interpretation of such yields, as crude products will often contain cells, which are bound to the polymer when it is recovered from fermentation broth[40,56]. The fermented broth has to be purified to obtain pure cellulose.

The most widely used process of purification of bacterial cellulose in the culture medium is the treatment with alkali (sodium hydroxide or potassium hydroxide), organic acids like acetic acid or repeated washing of the mixtures with the reverse osmosis water or hot tap water for a period of time[40,56]. Bacterial cellulose containing entrapped cells was treated with solutions like NaOH/KOH/Na_2CO_3 at 100 ºC for 15-20 min to lyse the microbial cells; thereafter the solution was filtered using an aspirator to remove the dissolved materials. The filter cake was repeatedly rinsed with distilled water until the pH of the filtrate became neutral. The dry mass of bacterial cellulose without any microbial cells was measured after drying for 4-6 h. As such, the dry cell mass was considered to be the difference between the mass of the dried bacterial cellulose containing the cells and the dried bacterial cellulose after the treatment with NaOH[62-65]. The culture medium was treated with acetic acid after the addition of NaOH solution for neutralization and then with distilled water[66,67]. The cells can be treated with aqueous solution of SDS and washed with aqueous NaOH, followed by neutralization with acetic acid or by repeated washing with distilled water and then drying in the air or at 60-80 ºC to a constant mass[62,68].

Properties of biofabric from bacterial cellulose: Bacterial cellulose as a nano fibers and biological nonwoven fabric possesses high crystallinity, high tensile strength, extreme insolubility in most of the solvents, mold ability and high degree of polymerization[69,70]. The thickness of cellulose fibrils is generally 0.1-10 mm, one hundred times thinner than that of cellulose fibrils obtained from plants with good shape retention. Its water holding capacity is over 100 times (by mass) higher. Microbial cellulose is far stronger than plant cellulose[71,72]. Macroscopic morphology of cellulose strictly depends on the culture conditions, which can easily be tailored for the physicochemical properties. Wanichapichart *et al.* demonstrated that cellulose fibre had the degree of polymerization of 793, with a corresponding molecular mass of *ca.* 142.73 kDa[73]. Cellulose is soluble in concentrated acids like sulphuric, hydrochloric or nitric acid. It is also soluble in 8.5 % NaOH solution. The solubility of cellulose in thealkali can be increased by adding 1 % of urea to the solution[74]. At higher temperatures (> 300 ºC) the biopolymer degrades, although the alkali-treated cellulose membrane is more stable (between 343 and 370 ºC). Composites prepared by adding bacterial cellulose and nanofibrillated cellulose (NFC) processed through fibrillation of raft pulp were compared for mechanical properties and it was found that the bending strength increased up to 425 MPa, while the Young's modulus increased from 19-28 GPa, nearly retaining the modulus of the bacterial

cellulose sheets[71,72]. The mechanical properties of cellulose are due to the uniqueness of uniform nano-scalar network structure, which is oriented bi-dimensionally when compressed[71].

Addition about bacterial cellulose, typically, networks of well-separated nano and microfibrils of bacterial cellulose create extensive surface area and hold a large proportion of water while maintaining a high degree of structural coherence. The water content of never-dried bacterial cellulose pellicles is *ca.* 99 % (w/w)[23,75]. A high density of inter- and intra-fibrillar hydrogen bonds offers a great deal of mechanical strength. The elastic modulus of dried bacterial cellulose is known to be around 15-30 GPa. Besides being chemically identical to plant cellulose, bacterial cellulose is produced in a virtually pure form free from hemicelluloses, pectins and lignin, which are present in plant cellulosic matrices. Moreover, the *in vivo* biocompatibility evaluation of bacterial cellulose in rats has demonstrated that it is well integrated into the host tissues and does not elicit any chronic inflammatory reaction, making it a potentially interesting scaffolding material for tissue engineering[75-77]. The unique physical and mechanical properties of bacterial cellulose as well as its purity can be exploited for multiple applications that range from high quality audio membranes, electronic paper and fuel cell to biomedical materials[23,24].

Application of biofabric from bacterial cellulose: The nanofibers and biononwoven of bacterial cellulose have properties like high purity, high degree of crystallinity, high density, good shape retention, high water binding capacity and higher surface area as compared to the native cellulose, it can be used in various areas including textile industry, paper, food, pharmaceutical, waste treatment, broadcasting, mining and refinery[23,78,79]. The applications of the bacterial cellulose are summarized in the following fields.

Bacterial cellulose (BC) has long been used in a variety of applications such as diaphragms in speakers and headphones[80], papermaking[81], separation membranes[82] and electro conductive carbon film[83]. Owing to its biocompatibility, bacterial cellulose has also recently attracted a great deal of attention for biomedical applications. For instance, bacterial cellulose has been successfully used as artificial skin for burn or wound healing material[22,23,35,84,85] artificial blood vessels for microsurgery[86]. The potential of bacterial cellulose scaffold for *in vitro* and *in vivo* tissue regeneration also continues to be explored and shows great promise[53,86-90]. To broaden the biomedical applications of bacterial cellulose, various attempts have been made to produce bacterial cellulose composites with high functionality[19,90-93]. Among them, BC/PEG composite is one of candidates that have great potential applications for tissue engineering and drug delivery. Bacterial cellulose to adsorb metal ions has been reported in the many previous studies[86].

In the biomedical area, bacterial cellulose can be used for wound healing applications[18], micro vessel endoprothesis[94], scaffolds for tissue engineered cartilage[89] and tissue engineered blood vessels[86]. Some of the materials based on bacterial cellulose, such as new skin substitutes and wound dressing materials, are now commercially available[23]. Other biomedical applications such as the use of bacterial cellulose as a regenerative aid to correct skeletal defects are under investigation.

Bacterial cellulose has been found to be attractive as a novel scaffold material due to its unique material properties. Porosity is the most important morphological parameter in the design of scaffolds for tissue engineering. Fabricating a scaffold with the desired pore size and porosity is of great importance in tissue engineering[95]. For bacterial cellulose scaffold, the definition of a specific pore size in a bacterial cellulose fibrous hydro gel is not relevant because the nanofibrils can be pushed aside by migrating cells[96]. Bacterial cellulose has potentialities to be an appropriate scaffold for different types of tissue and organ.

Microbial cellulose also has applications in mineral and oil recovery. There is a patented invention related to the use of bacterial cellulose in hydraulic fracturing of geological formations at selected levels of wells drilled for recovery of hydrocarbons. Addition of relatively small quantities of bacterial cellulose to hydraulic fracturing fluids improves their rheological properties and the friction through well casings is significantly reduced, resulting in lower pumping energy requirements. Computer models also indicate that formation fractures will be propagated for greater distances as will the propped portion of the fracture. Normally only *ca.* 0.60-1.80 g of bacterial cellulose per liter of fracturing fluid is needed[97]. Addition of cellulose nanofibrils obtained by acid hydrolysis of cellulose fibres at low concentrations to polymer gels and films as reinforcing agents showed significant changes in tensile strength and mechanical properties[98]. Based on the tensile strength, low oxygen transmission (barrier property) rate and its hydrophilic nature, the processed cellulose membrane appears to be of great relevance for its application as packaging material in food packaging, where continuous moisture removal and minimal oxygen transmission properties play a vital role[69]. The unique physical and mechanical properties of microbial cellulose such as high reflectivity, flexibility, light mass and ease of portability, wide viewing angles and its purity and uniformity determine the applications in the electronic paper display[79]. Fragmented bacterial cellulose has promising prospects in paper making, so test pieces of flexure-durable papers and high filler-content papers, which are ideal for banknote paper and bible paper, are being prepared[99].

Conclusion

Various methods for bio nonwoven fabric of bacterial cellulose production have been reported; some of which seem to demonstrate a potential tool for economic and commercial bacterial cellulose production: stationary culture, agitated culture, cultivation in the horizontal fermentors or cultivation in the internal-loop airlift reactors. The choice of a cultivation technique is strictly dependent on further biopolymer commercial destination. In the stationary culture conditions a thick, gelatinous membrane of bacterial cellulose is accumulated on the surface of a culture medium, whereas under agitated culture conditions cellulose can be produced in the form of a fibrous suspension, irregular masses, pellets or spheres. While stationary culture has been quite widely investigated and applied for production of some successful commercial cellulose products (Nata de Coco, transducer diaphragms, wound care dressing materials, *etc*.), agitated culture is still considered as a cultivation technique which is more suitable for the

commercial production of bacterial cellulose mainly due to the higher production rates which potentially can be achieved. However, it is also well known that cellulose production in fermentors with continuous agitation and aeration encounters many problems, including spontaneous appearance of cel- mutants (cellulose non-producers), which contributes to a decline in the polymer synthesis. Bacterial cellulose from Acetobacter strains displays unique physical, chemical and mechanical properties including high crystalline, high water holding capacity, large surface area, elasticity, mechanical strength and biocompatibility.

Microbial cellulose has proven to be a remarkably versatile biomaterial and can be used in a wide variety of fields, to produce for instance paper products, electronics, acoustics and biomedical devices. Various biodegradable and biocompatible polymeric materials have recently been investigated to fabricate inorganic-organic hybrid composites by mimicking the mineralization system of natural bone, with some successful outcomes. However, the search for an ideal biomaterial with properties and functionalities similar to natural bone is a continuing process because no single material can satisfy all the requirements for creating optimal scaffolding properties, such as strength, toughness, osteoconductivity, osteoinductivity, controlled degradation, inflammatory response and deformability. Degradation of bacterial cellulose has not been fully evaluated *in vitro* and *in vivo* settings. Other cellulose-based materials have however shown limited degradation. Although the complete degradability of materials for tissue engineering applications is very attractive, it is difficult to practically optimize and synchronize the degradation time and mechanical properties of the materials.

Among new commercial applications, bacterial cellulose has been shown to be very beneficial in the treatment of secondary and third degree burns. A clinical study has been performed on 34 patients. The bacterial cellulose wound dressing materials were directly applied on the fresh burn covering up to 9-18 % of the body surface. The following diagnoses were considered: macroscopic observation of the wound and wound extract, epidermis growth, microbiological tests and histopathological studies. Bacterial cellulose appears to be one of the best materials to promote wound healing from burns. Factors for this success include but are not limited to the following: a moist environment for tissue regeneration; significant pain reduction; specific cellulose nano-morphology which promotes cell interaction and tissue re-growth; significant reduction of scar tissue formation and, easy and safe release of wound care materials from the burn site during treatment. Microbial cellulose promises to have many new applications in wound care that extend beyond burn applications including, but not limited to, the following: surgical wounds, bedsores, ulcers, tissue, biotextiel, biological nonwoven fabric and organ engineering.

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