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Survey on Nano-fibers from Acetobacter Xylinum

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***Abstract*—**fibers of pure cellulose can be made from some bacteria such as *acetobacter xylinum*. Bacterial cellulose fibers are very pure, tens of nm across and about 0.5 micron 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 greatly 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.

***Keywords*—**Microbial cellulose, Biofabric, Microorganisms *Acetobacter xylinum*, Polysaccharide

I. Introduction

MICROBIAL cellulose is one of the most promising classes of microbial polysaccharides [1]-[8]. 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 [9], [10]. 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 [11], [12]. Exopolysaccharides are long chain polysaccharides consisting of branched, repeating units of sugars or sugar derivatives, mainly glucose, galactose and rhamnosein different ratios. They are classified into two groups: homopolysaccharides (cellulose, dextran, mutan, pullulan,curdlan), and heteropolysaccharides (gellan, xanthan) [13], [14].

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Cellulose is a linear polymer made of glucose molecules linked by (1--▸4) glycosidic linkages. 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 [15]-[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 *Gluconacetobactexylinus*, 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. BC 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]. Although synthesis of an extracellular gelatinous mat by *A. xylinum* was reported for the first time in 1886 by A. J. Brown, BC attracted more attention in the second half of the 20th century. Intensive studies on BC synthesis, using *A. xylinum* as a model bacterium, were started by Hestrin et al.(1947, 1954), 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 (C6H10O5)n is the same as that of plant cellulose, but their physical and chemical features are different [38],[39]. Fibers of bacterial cellulose can formed static and agitated cultures.

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 about 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 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 in 1949 [16],[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].

II. Biochemistry of Bacterial Cellulose

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 [46-49].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 MC 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 MC fibrils seems to bea key factor that determines its remarkable performances a wound healing system. Furthermore, the never dried cellulose membrane is a highly nano-porous 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, MC 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 [35],[55]-[61].

III. Some Properties Biofabric from Bacterial Cellulose

The thickness of bacterial cellulose fibrils is generally0.1–10 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 [62]-[65]. Microbial cellulose is far stronger than plant cellulose[66]-[70]. 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 approx. 142.73 kDa[71]-[73].

Cellulose is soluble in concentrated acids like sulphuric, hydrochloric or nitric acid. It is also soluble in8.5 % NaOH solution. The solubility of cellulose in thealkali can be increased by adding 1 % of urea to the solution [74].

At higher temperatures (>300 °C) the biopolymer degrades, although the alkali-treated cellulose membrane is more stable (between 343 and 370 °C). 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 to425 MPa, while the Young's modulus increased from 19to 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 about99% (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 around15-30 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].

IV. Some Application of Bacterial Cellulose

Bacterial cellulose (BC) has long been used in a variety of applications such as diaphragms in speakers and headphones [78-80], papermaking [81], separation membranes [82], and electro conductive carbon film [83]. Owing to its biocompatibility, BC has also recently attracted a great deal of attention for biomedical applications. For instance, BC 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 BC scaffold for in vitro and in vivo tissue regeneration also continues to be explored and shows great promise. To broaden the biomedical applications of BC, various attempts have been made to produce BC composites with high functionality [19],[39]. 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[23], scaffolds for tissue engineered cartilage [47] 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 [87]. 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. Bacterial cellulose has potentialities to be an appropriate scaffold for different types of tissue and organ[17].

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 [53]. 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 papermaking, so test pieces of flexure-durable papers and high filler-content papers, which are ideal for banknote paper and bible paper, are being prepared [88],[89].

V. Conclusion

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. In this study, the ultrafine 3-D BC network structure with its native unique properties is exploited for the synthesis of materials analogous to natural bone. Our study showed that the formation of apatite is dependent on the presence and type of surface functional groups in the microfibrillar BC network.

Among new commercial applications, BC 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 BCwound 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. BCappears 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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