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Morphology and Properties of Bacterial Cellulose: Influence of Various Growth Parameters on Structural and Funtional Properties

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ABSTRACT

Bacterial cellulose (BC), a superior form of cellulose synthesized by various microbial genera, offers significant advantages over plant-derived cellulose due to its unique properties such as high crystallinity, excellent water-holding capacity, mechanical strength, and moderate biocompatibility. It is widely used in medical applications, such as wound dressings, tissue engineering and in dentistry, it is explored for guided tissue regeneration, dental implant coating, enhancing tissue integration and healing. Bacterial cellulose morphology is influenced by various factors including the microbial species, synthetic pathways, culture conditions and culture methods. Typically, it takes the form of a gelatinous membrane, while agitated or shaking culture methods yield fibrous networks. Bioreactor cultures offer controlled environment that enhance fiber length, diameter, alignment, and overall mechanical properties. The crystalline nanofiber network of BC directly influences its properties such as tensile strength, water retention, and elastic modulus. The choice of cultivation method and cultural conditions significantly impacts the morphology and properties of BC, enabling customization for specific needs and promoting its adoption in fields such as healthcare, biotechnology, and sustainable manufacturing.

Keywords: *Bacterial cellulose, Nanofibers, Crystallinity, Bioreactor Cultures, Static Cultivation, Agitated Cultivation.*

1. INTRODUCTION

Cellulose is the most abundant biopolymer on earth, primarily found in plant cell walls. Its abundance and structural integrity make it a crucial component in providing strength and support to plant cells, allowing them to maintain their shape and withstand environmental stresses.

Additionally, certain bacteria, algae, and some animals like tunicates also produce cellulose.[1]

Bacterial cellulose (BC) is a remarkable form of cellulose that offers several advantages over plant-derived cellulose. The first report of cellulose produced from bacteria, specifically from *Acetobacter xylinum,* was announced by Brown in 1886. Later studies have revealed that cellulose can be produced by different bacteria, including Gram-negative bacteria species such as *Acetobacter Azotobacter, Rhizobium, Agrobacterium, Pseudomonas, Salmonella, Alcaligenes*, as well as Gram positive bacterial species such as *Sarcina ventriculi*. [2,3]

BC boasts exceptional physical and mechanical properties, such as high crystallinity, superior water-holding capacity, mechanical strength, and moderate biocompatibility that surpass those of plant-derived cellulose. These qualities make BC highly desirable for various applications, including wound care, drug delivery, tissue engineering and in dentistry it can be used as dental implant coatings, guided tissue regeneration membranes, dental scaffolds for bone and pulp regeneration, and

root canal filling materials.[3] However, despite its inherent advantages, BC lacks certain functionalities like antibacterial or magnetic properties, limiting its application scope. To address this, extensive research has focused on modifying BC's structure through various chemical entities, resulting in enhanced properties. [4]

Understanding the morphology of bacterial cellulose is essential for comprehending its structure-function relationships, while the significance of modification lies in enhancing its properties for tailored applications. [5]

The present overview discusses the cellular morphology and properties of bacterial cellulose and the various factors influencing it.

BACTERIAL CELLULOSE SYNTHESIS

The synthesis of bacterial cellulose is a natural process driven by specialized bacterias, resulting in a pure and highly crystalline form of cellulose with unique physical characteristics.[6] Glucose is the primary substrate for BC synthesis, although alternative sugars like fructose and galactose can also be converted into cellulose through various metabolic pathways.[7]

UDP (uridine diphosphate) serves as the precursor for bacterial cellulose (BC) synthesis, which is produced by microbial cells in a four-step process. Firstly, monosaccharides are activated through glucose nucleotides, initiating the pathway for cellulose synthesis. This activation step primes the monosaccharides for polymerization. Subsequently, glucose units polymerize to form cellulose chains in a process facilitated by enzymes within the microbial cells. The polymerization of glucose units results in the formation of long β-(1→4) glucan chains.[8]

Following polymerization, an acyl group is added to individual glucose units, contributing to the structural stability of the cellulose chains. This acylation process enhances the mechanical strength of the resulting BC. Once the β-(1→4) glucan chains are formed and acylated, polymerization continues, integrating thousands of individual chains into fibrils.[8]

In a study conducted by Wang et al, BC producing bacteria *Komagataeibacter sp. W1*, was subjected to media spiked with various carbon sources including acetate, ethanol, fructose, glucose, lactose, mannitol, and sucrose. After 14 days of incubation at 30°C, the BCs were collected and the highest yield being observed in fructose.[8] Mikkelsen et al. investigated the effects of six carbon sources, glucose, glycerol, mannitol, fructose, sucrose, and galactose on bacterial cellulose (BC) production by *Gluconacetobacter xylinus*. They found that sucrose yielded the highest BC production at 3.83 g/L, followed by glycerol, mannitol, glucose, and fructose, which were effectively transported across the cell membrane. Galactose was the least suitable carbon source, as its transformation to cellulose was inefficient due to poor uptake by the bacteria.[9]

Similar to yield, the properties of bacterial cellulose are also influenced by factors such as substrate and bacterial strain.[9]

CHEMICAL STRUCTURE OF BACTERIAL CELLULOSE:

BC consist of a linear homopolymer of glucose monomers linked by glycosidic linkage with the chemical formula $(C_6H_{10}O_5)_n$. Here, two successive glucose monomers are linked in a manner where the former glucose unit is rotated at 180 degrees with reference to the preceding one.[10]

Even though both plant cellulose and bacterial cellulose have the same chemical composition, BC represents the purest form of cellulose, due to the absence of lignin, pectin, and hemicelluloses.[11] The degree of polymerization of BC typically ranges between 14000 - 16000, which is higher compared to plant cellulose ranging from 300 - 10000. A higher DP means longer polymer chains, which often leads to improved mechanical strength, stability, and durability of the cellulose structure. Hydrogen bonds are plentiful within cellulose due to the presence of a large number of oxygen atoms and hydroxyl groups. Parallel stacking is observed within cellulose due to the presence of van der Waals force that helps in the development of crystalline nanofibers followed by the development of microfibrillar structure. The presence of the supercoil structure helps in promoting the hierarchical orders that help in providing a very high amount of mechanical strength.[12]

Chemical structure analysis using techniques like Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) has revealed slight variations in BC produced by different methods and from different sources. Structural analysis by NMR has shown that BC produced by microbial and cell-free systems demonstrate cellulose I and cellulose II polymorphic structures, respectively. Similarly, X-ray diffraction (XRD) analysis has confirmed these polymorphic structures.[13]

Cellulose I, also known as native cellulose, is the naturally occurring cellulose, which consists of parallel strands without inter sheet hydrogen bonding, primarily found in the plant cell wall. Cellulose II is thermodynamically more stable and exists in antiparallel strains with inter sheet hydrogen bonding. Cellulose II does not exist naturally, but can be obtained from the native material by mercerization which involves swelling treatment with sodium hydroxide.[16] Cellulose I can be subdivided into two amorphous forms: cellulose Iα and cellulose Iβ. Cellulose Iα, the less stable form, has a triclinic unit cell, while cellulose Iβ, the more stable form, features a monoclinic unit cell. When cellulose fibers are produced by bacteria, their orientation is random, leading to an alternation between these two phases within the overall cellulose structure. Cellulose I exhibit high crystallinity and low solubility, while cellulose II has lower crystallinity and higher solubility.[14]

Various analytical techniques such as CP/MAS 13C NMR spectroscopy, wide-angle X-ray diffraction, and transmission electron microscopy (TEM) have been employed to study the solid-phase nitration and acetylation of BC.[6] **Solid-phase nitration and acylation of bacterial cellulose** are chemical processes that modify properties and used for structural studies

of bacterial cellulose (BC) by introducing new functional groups (acetyl and nitro groups) in a controlled, step-wise manner while the cellulose remains in its solid state.[14]

PHYSICAL STRUCTURE OF BACTERIAL CELLULOSE:

The physical structure of BC is noteworthy, consisting of a three-dimensional network of nanofibers. These nanofibers form a hydrogel sheet. The celluloses produced by different bacteria possess different morphology. *A. hansenii, Aerobacter and Agrobacterium* form fibril structure, *Acetobacter* and *Achromobacter* form ribbon like structure, *Gluconacetobacter* form 3-D network nanofibers and *Pseudomonas* form amorphous/not defined structure of BC.[9]

A study on *Gluconacetobacter xylinus* bacteria by William et al confirmed that cellulose crystallization occurs serially following its secretion along one side of the cell, leading to a cellulose ribbon that can reach several micrometers in length and combine with ribbons from other cells to form a robust biofilm matrix.[15] The surface analysis of bacterial cellulose (BC) produced by Gluconacetobacter hansenii, as revealed by scanning electron microscopy (SEM), shows a random fiber distribution, while cross-section analysis showed layers of clustered fibers. Atomic Force Microscopy (AFM) images of bacterial cellulose (BC) revealed its reticulated structure. The diameters of the microfibril, bundle, and ribbon were 17 nm, 37 nm, and 62 nm, respectively. The nascent chains of BC aggregate into fibers and ribbons through multiple steps, forming a porous structure with a high aspect ratio, which increases the surface area of BC. The nanoscale diameter of BC fibers supports its classification as nanocellulose compared to cotton fibers. These microstructural features are key to the unique and promising properties of BC.[16] The small diameter and high aspect ratio of bacterial cellulose fibers collectively contribute to its increased hydrophilic properties[17]

The fiber thickness of bacterial cellulose (BC) also varies with the bacterial strain used for production. For instance, *Acetobacter xylinum* (now reclassified as *Gluconacetobacter xylinus*) typically produces fibers ranging from 50 to 100 nanometers in thickness. In contrast, *Gluconacetobacter hansenii* generates fibers approximately 50 to 80 nanometers thick,[9] while *Gluconacetobacter kombuchae* produces fibers generally around 50 to 70 nanometers in thickness.[9] The unique structure of BC imparts remarkable physicochemical properties, including high surface area, porosity, and water-holding capacity. The entanglement of nanofibers efficiently distributes stress throughout the material, and the dense, well-organized fiber network contributes to a high elastic modulus.[17]

The **morphology** and **fiber thickness** of bacterial cellulose are significantly influenced by the cellulose synthase operons, particularly the **bcs operon**. Disruptions in specific operons can reduce cellulose production or cause irregular fiber formation.[18]Genetic modification, though not extensively researched, offers potential benefits for enhancing bacterial cellulose yield and properties.Modification of **Komagataeibacter's** genetic material to reduce harmful mutations, enhanced cellulose production, and improved cellulose properties like mechanical strength, porosity, and crystallinity for specific applications.[19]

The **size and diameter of bacterial cellulose (BC) fibers** also depend on the growth conditions, especially the carbon source in the culture medium. According to Kiziltas et al. (2015), BC produced using **wood sugar** as a substrate resulted in **smaller diameter fibers** compared to those grown in the standard **Hestrin-Schramm (HS) medium**.[20] Chen et al. (2019) demonstrated that the **number of cellulose layers** correlate with the **size of the fiber,** more layers result in larger fibers.[21] Betlej (2019) found that bacterial cellulose produced on a **nitrogen-rich substrate** had **multilayered** structures and greater surface **corrugation,** compared to cellulose grown in nutrient-poor media.[22]

Mechanically, BC exhibits impressive properties. The Young's modulus of BC sheets typically ranges from 16 to 18 GPa, with potential for further improvement up to 30 GPa. Notably, BC demonstrates exceptional strength along the perpendicular direction of fiber growth, highlighting its structural integrity and potential for load-bearing applications.[9] BC has a high degree of crystallinity, typically ranging from 60% to 90%, which contributes to its exceptional tensile strength and stiffness due to the densely packed and highly ordered crystalline regions providing robust intermolecular hydrogen bonding. Additionally, cellulose dried using the **lyophilization method** showed a crystallinity of **91.6%.**[9]

The **porous structure** of bacterial cellulose allows excellent **gas exchange**, facilitating faster tissue regeneration after surgery compared to conventional synthetic materials. Its dense **nanofiber network** acts as a barrier, preventing the penetration of infectious agents while maintaining a moist, biocompatible environment that supports healing. This combination of properties makes bacterial cellulose highly effective for medical applications like wound dressings and tissue scaffolds. Additionally, its high permeability allows for the controlled transfer of drugs to the affected tissue. [22]

PROPERTIES OF BACTERIAL CELLULOSE IN DIFFERENT CULTURE METHODS:

Various methods are used to prepare bacterial cellulose (BC), including static cultures, agitated or shaking cultures, and bioreactor cultures. Each method produces BC with different macroscopic morphology, microstructure, and properties. For instance, static culture yields a gelatinous membrane of cellulose on the surface of the nutrient solution, while agitated or shaking culture leads to asterisk-like, sphere-like, pellet-like, or irregular masses of BC.[23]

The condition of the culture environment, including bacterial strain, nutrition, pH, temperature and oxygen delivery, is also crucial and impacts the properties as well as yield of BC.[20,24] One of the most important parameters is the temperature. A temperature range of 25 to 30 °C was found to be best for the production of BC, as a *Komagataeibacter sp*. was cultivated

at 30 °C for 7 days under static conditions and temperature of 33.5°C was required by *Acetobacter senegalensis* MA1.[9]

Stanisławska et al. (2020) compared the quality of the polymer dried at 25 °C and 105 °C, found that the tensile strength of cellulose dried at 25 °C was 17.5 MPa and was 15 times higher than the strength of cellulose dried at higher temperature and proved that the drying temperature of a polymer has a significant impact on its strength.[25]Indriyati et al. (2019) suggested that the method of handling the polymer after the completed cultivation period, in particular, treatment with alkali, improves its mechanical properties.[26] The treatment of cellulose with NaOH and NaClO has been shown to increase its Young's modulus to as much as 30 GPa.[27]

pH is another important factor in controlling oxidative fermentation of BC production. Acidic or near-neutral pH is suitable for BC. pH 4–6 is considered the ideal pH for the fermentation culture medium of BC. Experimental observations indicate that pH of 5.50 for *Acetobacter xylinum*, and 6.0 for *Komagataeibacter* spp.are required. Extreme pH values can disrupt cellulose synthesis pathways, resulting in lower crystallinity or changes in polymerization. [14,20] Crystallinity is also affected by the substrate used.[22] Yim et al (2017) proved that, depending on the type of carbon source, the degree of crystallinity of bacterial cellulose can range from 13 to 74%.[28] Research has shown that the integration of alginate into bacterial cellulose (BC) structures greatly boosts their water-holding capacity. Due to its hydrophilic properties, alginate forms hydrogen bonds with water molecules, enhancing the material's moisture retention ability.[22]

Bacterial cellulose can also be synthesized from waste materials and naturally abundant resources to reduce production costs. The fermentation condition and type of substrate had impact on some of the structural and physicochemical properties. Akintunde et al cultured *Komagataeibacter* in sugarcane bagasse and corncob **hydrolysate media**, produce a densely packed cellulose network with thinner fibers and a more compact pattern. They exhibited higher **thermal stability** compared to those produced in the defined **HS medium**. However, BC from the HS medium showed superior **mechanical properties**, with a high **modulus of elasticity** and strong **tensile strength.[29]**

BACTERIAL CELLULOSE BY STATIC FERMENTATIVE CULTIVATION:

Static fermentative cultivation is a widely utilized method for producing bacterial cellulose (BC) sheets, films, or membranes. In static cultivation, BC is formed at the air-medium interface and adopts the shape of the container in which it is produced. As new cellulose fibrils are synthesized, the thickness of the BC gradually increases, depleting the available nutrients in the medium and eventually leading to the inactivation of the bacterial cells as they become entrapped within the BC matrix.[24] This method is particularly suitable for applications in the biomedical field, where a reticulated structure obtained through static cultivation is often required for scaffolds and tissue engineering purposes. The structure is more **porous** and less densely packed. [29]

BC from **static culture** has **more stable properties,** making it a preferred method for production. Due to its simplicity and widespread use, **static culture** remains a common approach for BC production.[22] High cost and low rate of production are the two main problems in static culture systems. To solve these problems, use of an agitated/shaking culture has been suggested.[22]A **modified static culture** using an **intermittent feeding strategy** allowed for the production of BC films with **arbitrary thickness** in a layer-by-layer form. By adding nutrients directly on top of the formed BC, microorganisms could access both oxygen and nutrients, maintaining a **steady production rate**. This approach resulted in a BC thickness of approximately **30 mm** after 30 days, compared to just **2 mm** in conventional static culture and BC products can be obtained with arbitrary shape and thickness continuously. (Wang et al,2016).[30]

BACTERIAL CELLULOSE BY SHAKING/ AGITATION FERMENTATIVE CULTIVATION:

Bacterial cellulose (BC) obtained through shaking fermentative cultivation presents distinct structural characteristics and properties compared to BC derived from other methods. Typically, BC produced via shaking culture retains a fibrous network structure akin to that of statically cultivated BC.[19] Here oxygen is continuously mixed into the medium, so the BC is produced with enhanced yield compared with static culture, which contributes to cost reduction.[21]

However, the agitation introduced during shaking fermentation can influence the arrangement and organization of cellulose fibrils within the BC matrix. This agitation fosters the formation of BC pellets of diverse sizes and shapes, impacting the internal structure and porosity of the BC matrix.[31] The shape and size of these pellets are contingent on various factors including shaking speed, microbial strain type, and incubation conditions.[32]

Zywicka et al studied the effect of various agitation modes on BC synthesis by different strains of *Gluconacetobacter xylinus* and result shows that higher density of *G. xylinus* cells were found in the culture subjected to a higher stirring speed. It was also determined that the highest weight of BC was obtained in the culture agitated at the speed of 150 rpm. It has also been shown that with an increasing stirring speed, the synthesized BC was characterized by more irregular shapes.[31]

BC produced by agitated culture displays some changes of microstructure and properties, such as a low degree of polymerization, a low crystallinity index, and inferior mechanical properties.[22,23] The microstructure of the spherelike BC is quite different from that produced by other culture methods.[31]

Despite the agitation, BC maintains its high water retention capacity.[12] **Agitated culture** of *Komagateibacter medellinensis*

using grape pomace (GP) resulted in **higher BC production yield** compared to **static culture**. The BC produced under agitation formed **sphere-like structures.** Additionally, the **water holding capacity (WHC)** of the BC was significantly higher in the agitated culture, showing a **60% increase** compared to BC membranes from static culture, due to the **superabsorbent nature** of the spherical BC (Ramirez et al, 2021).[33]

Moreover, BC obtained through shaking fermentative cultivation upholds its inherent biocompatibility and biodegradability. The absence of toxic components and its natural origin contribute to BC's biocompatibility, while its enzymatic degradation in biological environments underscores its biodegradability.[22]

BIOREACTOR CULTURES:

The production of BC in the agitated culture is reduced due to the significant growth of cellulose-negative mutants, accumulation of unwanted acids, and adhesion of BC broth to the wall of the reactor. Therefore, to overcome these limitations, bioreactors were developed which can increase productivity, reduce production cost and shorten the cultivation time.[9]

Bioreactor cultures are a method used for the production of bacterial cellulose (BC). In these cultures, bacteria are grown in a controlled environment within a bioreactor, allowing for precise regulation of parameters such as temperature, pH, and nutrient availability. Based on the various fermentation purposes, to increase the BC production, different bioreactors have been designed including stirred tank reactors, airlift, aerosol, membrane reactors, and other types.[34] Airlift bioreactors have been widely used in biochemical processes due to their simple design and ease of maintenance. It also has many advantages including lower shear stress, less energy consumption and high oxygen transfer rate.[35] Airlift bioreactor produces a membrane-type BC from Gluconacetobacter xylinus, the water-holding capacity of which is greater than that of cellulose types produced using static cultivation methods. The Young's modulus of the product can be manipulated by varying the number of net plates in the modified airlift bioreactor (Wu etal,2015).[36]

Bioreactor cultures often promote the formation of BC with more uniform and organized structures, leading to enhanced morphological properties such as increased fiber length, diameter, and alignment. This can result in BC with improved mechanical properties, such as tensile strength and elasticity. Additionally, bioreactor cultures may minimize the presence of impurities and non-cellulose materials, resulting in BC with higher purity and smoother surface morphology. Overall, the controlled conditions provided by bioreactors can lead to more consistent and desirable cell morphologies in bacterial cellulose.[34]

CELL FREE SYSTEM:

Recent advancements have introduced a novel approach to BC production utilizing cell-free enzymatic systems, offering potential advantages over conventional cellular methods. The cell-free enzyme system is developed from BC-producing strains and contains whole enzymes and cofactors required for BC synthesis.[9] It produces pure cellulose with similar chemical composition (β-1,4-glucan chains), crystallinity, and mechanical properties as the conventional method. However, it yields highly pure cellulose by eliminating bacterial cells and by-products, and offers precise control over the synthesis process, allowing customization of fiber morphology, size, and thickness by adjusting conditions.[37]

The properties of bacterial cellulose can be significantly enhanced through **modification** by blending it with other polymers. Bacterial cellulose (BC) composites are materials created by combining BC with other substances to enhance its properties and expand its applications. **Chitosan**, a biocompatible and antibacterial polysaccharide derived from chitin, is modified with BC to create **BC-chitosan composites**, widely used in the treatment of burns, skin ulcers, bedsores, and wounds, providing an effective environment for healing while also offering antibacterial protection. The combination of BC with **polyvinyl alcohol (PVA)** results in composite materials that leverage the beneficial properties of both substances. PVA is a hydrophilic, water-soluble synthetic polymer, nontoxic and boasts excellent thermal stability, transparency, and high mechanical strength.[38]The **nanoporous structure** of bacterial cellulose, which contains nanofibers, offers a significant amount of sub-micron pores for hosting metal nanoparticles.[38]These nanoparticles can be embedded within the BC porous network. BC based nanocomposites has excellent structural and physical properties such as high surface area, special surface chemistry, high crystallinity, mechanical strength, hydrophilicity, and excellent biological features (biocompatibility, biodegradability, and non-toxicity) and can used for wound dressing, drug delivery etc.[39]

Research and development continue to explore new BC composite formulations and applications, driving innovation in sustainable materials science.

2. CONCLUSION

BC stands out as a remarkable biomaterial with diverse applications across various industries. Its unique properties, including high biocompatibility, mechanical strength, water retention capacity, and moldability, make it an attractive choice for fields such as biomedical engineering, tissue regeneration and in dentistry, it can be used as dental implant coating, guided tissue regeneration membrane, dental scaffolds for bone and dental pulp regeneration, root canal filling material etc. Despite its

inherent advantages, BC also faces limitations, such as the lack of specific functionalities like antimicrobial or magnetic properties.

The cultivation methods and characteristics for BC production play a crucial role in determining its cellular morphology, properties and applicability. Static and shaking fermentative cultivation methods offer unique advantages and challenges, allowing for the customization of BC properties based on application requirements, production scalability, and resource availability. Overall, bacterial cellulose represents a promising biomaterial for future innovations, with ongoing research and innovation driving its evolution and expanding its utility in a myriad of domains. As advancements continue, BC is poised to emerge as a versatile and indispensable material in various industries, contributing to advancements in healthcare, biotechnology, and sustainable manufacturing.

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