

Review

BACTERIAL CELLULOSE: A NEW BARRIER MEMBRANE IN IMPLANTOLOGY

*Abu Nazar N, ** Sheejith.M, *** Jinsha Maji K, ****Swapna C, *****Nila S Das

Abstract

Bacterial cellulose (BC), synthesized by Gram-negative bacteria like *Gluconacetobacter xylinus*, showcases a distinctive cellular morphology. This morphology involves a 3D network of uniaxially oriented cellulose nanofibers, with diameters of 3 to 8 nanometers, contributing to heightened crystallinity (60-80%). BC's architecture is formed through glucose polymerization, resulting in linear glucan chains that assemble into ribbon-like structures. This unique cell morphology grants BC exceptional mechanical strength, porosity, and biocompatibility. Understanding BC's cellular arrangement offers insights into its structure-property relationship, shaping its diverse applications in dentistry such as barrier in implantology, guided tissue regeneration membrane, dental scaffolds for bone and dental pulp regeneration. By harnessing the structural marvel of bacterial cellulose, scientists and engineers are poised to create novel materials that bridge the gap between nature and technology, fostering sustainable solutions for a variety of industries.

Keywords: Bacterial cellulose, Nanofibers, Crystallinity, Glycosidic bonds, Protofibrils

Corresponding Author: Dr Jinsha Maji K

*** Post graduate student, * Associate Professor, ** Professor and Head, **** Professor, ***** Assistant Professor Department of Prosthodontics-Crown & Bridge, KMCT Dental College, Kozhikode, Kerala.

Introduction

Cellulose is one of the most abundant and widely distributed organic polymers on Earth. Its presence in various organisms, including green plants, algae, and certain microorganisms, underscores its

fundamental role in biological structures.¹

As a polysaccharide composed of repeating glucose units connected by $\beta(1\rightarrow4)$ glycosidic bonds, cellulose forms long, linear chains that contribute to the structural

integrity of plant cell walls and other cellular components.²

There are various types of cellulose like bacterial cellulose, cellulose acetate, ethyl cellulose, and hydroxypropyl cellulose

Bacterial cellulose (BC) is a remarkable form of cellulose.³ It is a unique biomaterial that has gained attention in various medical and tissue engineering applications, including those related to dental and oral health.⁴ Its properties, such as biocompatibility, biodegradability, and the ability to be molded into various shapes, make it suitable for use in these applications.

In implantology, bacterial cellulose-based barrier membranes are typically used in guided bone regeneration (GBR) procedures. These procedures aim to regenerate lost or damaged bone and soft tissues around dental implants or in areas affected by periodontal disease. The barrier membrane acts as a physical barrier to prevent the migration of epithelial cells into the defect site, allowing space for bone and soft tissue regeneration.⁵

Because of these advantages, research into bacterial cellulose and its applications continues to grow, offering exciting

opportunities for various industries looking for innovative and sustainable materials.²

The present overview discusses the cellular morphology of bacterial cellulose.

BACTERIAL CELLULOSE:

Bacterial cellulose is produced by certain Gram-negative bacterium *Gluconacetobacter xylinus* (conventionally known as *Acetobacter xylinum*) .*Agrobacterium* spp., *Acetobacter* spp., *Azotobacter*, *Rhizobium* spp., *Sarcina*, *Alcaligenes*, and *Pseudomonas* aerobic bacteria as an extracellular polysaccharide membrane in a bottom-up approach. The final result is pure cellulose consisting of a 3D network of nanofibers.²

BC can be obtained from bacterial cultures (*Hestrin-Schramm* (HS) Medium, *Yamanaka* Medium, *Zhou's* medium , molasses and date syrup) in many formats and for different purposes, such as membranes under static culture conditions, and as micro- or nano-structures in shaken cultures.⁵ 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.

Anselme Payen, a French chemist, is indeed credited with the discovery of cellulose. In 1838, Payen successfully isolated cellulose from plant material and conducted chemical analyses to characterize its properties.⁶ The production of cellulose from bacterial sources was indeed first reported by Brown in 1886. He observed the ability of the Gram-negative bacterium *Komagataeibacter xylinus* (previously known as *Gluconacetobacter xylinus*) to synthesize cellulose.⁷

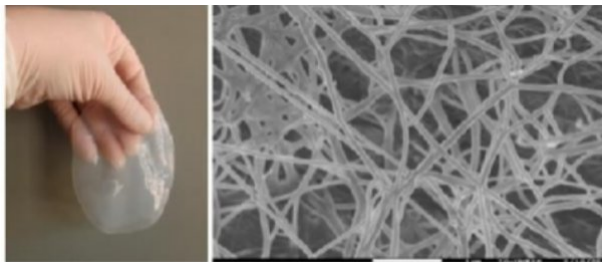


Figure 1 : Bacterial cellulose

Because of its uniform structure and morphology, the BC is endowed with peculiar characteristics, such as a high water retention capacity, high purity and crystallinity, good chemical stability, and remarkable mechanical properties⁸

Bacteria produce BC through a cellulose synthase complex, an integral membrane protein that catalyzes the chemical addition reaction of glucose units.

In general, the glucose polymerization process involves:

Activation of Cellulose synthase reaction by cyclic diguanyl monophosphate (c-di-GMP)



Polymerization of single glucose residues into linear β -1,4-glucan chains



Extracellular secretion of linear glucan chains



Crystallographic assembly of a single ribbon of fibrils containing up to 1000 glucan chains

BC fibrils are condensed in a network located in the interphase of the culture media-air. The macroscopic aspect of the BC membrane is similar to a gel, whose form and thickness depend on the recipient and cultivation time, respectively.⁹

CELLULAR MORPHOLOGY OF BACTERIAL CELLULOSE:

Bacterial cellulose is composed of an intricate network of cellulose nanofibers,

which are extremely fine with diameters ranging from 3 to 8 nanometers. These nanofibers are notably uniaxially oriented, meaning they align in a single direction and a branched network is formed of BC fibrils connected to each other, in contrast to the plant cellulose, the fibers of which have a significantly greater thickness and lower density of hydrogen bonds.¹⁰ During synthesis, protofibrils of glucose chains are secreted through bacterial cell walls, aggregating to form nanofibrils and ribbons. These ribbons are exceptionally thin, measuring approximately 3–4 nanometers in thickness and 70–80 nanometers in width.¹¹ These ribbons intertwine and create a web-shaped network within the bacterial cellulose matrix. This network structure contributes to BC's mechanical strength and porous nature.¹²

The SEM image of raw BC sheet demonstrates a lot of small holes equally

distributed on the surface, and many slight entanglements exist between these

holes. When magnifying 10000 times, the surface is almost flat and smooth.¹³

During BC production by bacteria like *Acetobacter xylinum*, both cellulose I and cellulose II polymorphs are produced.

Cellulose I is the initial ribbon-like form of cellulose that the bacteria secrete. Over time, some of this cellulose I undergoes transformation into cellulose II, which is more thermodynamically stable.¹⁴

The surface structure of bacterial cellulose (BC) film is characterized by a remarkable three-dimensional fibrous network composed of nanofibrils that are randomly arranged and held together by hydrogen bonds within the BC fibril units. This network exhibits a complex architecture, with numerous intertwined strings creating a mesh-like structure, formed by the overlapping fibrils, resulting in a layer of BC ribbons oriented in a random fashion. These ultrafine BC ribbons, which typically range in length from 1 to 9 μm , come together to construct a dense reticulated structure that is stabilized by an extensive network of hydrogen bonds. Moreover, the degree of polymerization value of BC falls within the range of 2,000 to 6,000 and can even reach as high as 20,000, highlighting the substantial molecular complexity and unique structural characteristics of this biopolymer.¹¹

The field emission scanning electron micrograph (SEM) reveals a fascinating microstructure characterized by a

reticulated pattern composed of ultra-fine cellulose fibrils. These fibrils exhibit a unique entangled and curved arrangement, contributing to the formation of a dense, reticulated structure. Notably, during static production, the cellulose fibers extend significantly, accentuating the complexity of this natural material. This intricate assembly of fibrils culminates in a porous structure boasting an exceptionally high aspect ratio, signifying a substantial surface area relative to its volume.

Bacterial cellulose (BC) undergoes a multi-stage transformation, starting with the aggregation of nascent chains into subfibrils. These subfibrils subsequently undergo crystallization, evolving into microfibrils, which then bundle together to form ribbons. This hierarchical assembly underscores the remarkable versatility of cellulose in building complex structures from the nanoscale to the microscale.¹³

CRYSTALLINITY OF BACTERIAL CELLULOSE:

The oriented nanostructure of bacterial cellulose results in a higher degree of crystallinity compared to other forms of cellulose.¹³ Crystallinity is the measure of how organized the cellulose chains are, and in bacterial cellulose, it can reach levels of

60-80% contributing to material's exceptional mechanical and physical attributes.¹⁴

The crystal structure of native cellulose is referred to as cellulose I. In cellulose I, all the cellulose strands are oriented in a parallel fashion, and there is an absence of inter-sheet hydrogen bonding within the structure. It's important to note that crystalline native cellulose isn't a single, uniform structure but rather a combination of two distinct crystalline modifications, namely cellulose Ia and cellulose Ib.¹⁴

Bacterial cellulose are known to contain a significant proportion of Ia modification. This means that in these types of cellulose, the cellulose Ia crystalline form predominates. The presence of different crystalline modifications can affect the material's properties and behavior, making it important to understand and distinguish between these structural variations in cellulose for various applications.¹⁶

The cellulose Ia phase is characterized by a triclinic crystal structure with a P-I space group. In this structure, there is only one cellulose chain per unit cell. On the other hand, the cellulose Ib modification has a monoclinic crystal structure, belonging to

the Meyer-Misch type, with a space group of P-21.¹⁷

Figure 2 :TYPES OF CELLULOSE BY DIFFERENT MICROORGANISMS: [18]

BACTERIA	CELLULOSE
Acetobacter	Extracellular pellicle Cellulose ribbons
Agrobacterium	Short extracellular fibrils
Pseudomonas	Short extracellular fibrils
Rhizobium	No distinct fibrils
Sarcina	Amorphous fibrils

The cell morphology of cellulose produced by Acetobacter bacteria typically consists of long, thin, ribbon-like structures. These cellulose ribbons are microscopically visible and can vary in dimensions, but they are generally characterized by being much thinner and more elongated fibres typically ranging in width from 40 to 60 nanometers. [19]This ribbon appears to be oriented parallel to the longitudinal axis of the bacterial cell. Cellulose ribbons produced by Acetobacter bacteria are a result of extracellular cellulose biosynthesis. Even though A. xylinum is nonmotile, it can be propelled by the formation of this cellulose ribbon, at a rate of approximately 2 micrometers per minute.²⁰

Agrobacterium tumefaciens is not typically known for cellulose production. Instead, it is more renowned for its role in genetic transformation of plants, leading to the formation of plant tumors (crown galls). The cellulose fibrils produced by Agrobacterium tumefaciens likely serve as an extracellular matrix that surrounds the bacterial cells. This matrix can play a role in various cellular processes, including adhesion to host plant cells, biofilm formation, and the establishment of infections. They produce short extracellular fibrils.²¹

Pseudomonas is a genus of bacteria known for its diversity and adaptability to various environments. Some species of Pseudomonas have been found to produce cellulose, without distinct fibrils.²²

Conclusion

Bacterial cellulose stands as a remarkable organic polymer with a distinct cellular morphology, harnessed from Gram-negative bacteria like Gluconacetobacter xylinus. The intricate 3D arrangement of uniaxially oriented cellulose nanofibers confers exceptional properties, including water retention, crystallinity, mechanical strength, and stability. Orchestrated by the cellulose synthase complex, glucose units

are assembled into ribbon-like structures that intertwine to form a robust network within the bacterial cellulose matrix. With its sustainable nature and innovative properties, bacterial cellulose holds a promising future as a versatile biomaterial, offering exciting prospects for further advancements and novel industrial applications.

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