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Perspective

Three-Dimensional Hierarchical Cellulose Structures Based on Microbial Synthesis and Advanced Biofabrication

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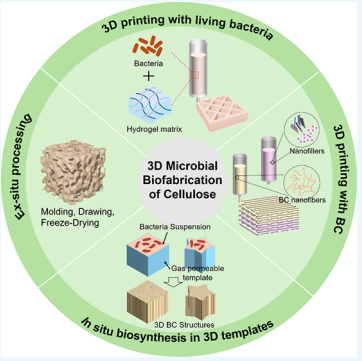
ABSTRACT: Cellulose is the most abundant and important biopolymer in our world, and it can also be biosynthesized by certain types of bacteria, such as *Komagataeibacter xylinus*. However, due to the requirement of oxygen access during such bacterial cellulose (BC) biosynthesis, as well as the high crystallinity and poor processability of BC, it is very challenging to fabricate 3D BC structures with well-defined shape, geometry, and internal structure. In recent years, the rapid progress of polymer additive manufacturing and biofabrication has provided new and versatile approaches for fabricating hierarchical 3D cellulose structures. This can be achieved by either incorporating BC in the 3D printing feedstock or, more interestingly, by incorporating cellulose-generating bacteria in a living ink followed by in situ BC biosynthesis. In this Perspective, we critically examine the potential of various advanced biofabrication technologies in fabricating hierarchical 3D cellulose structures, especially those based on integrating additive manufacturing with in situ microbial biosynthesis. Moreover, sustainable biocomposites based on BC and microbial biosynthesis are also discussed. The current challenges and future opportunities of microbial-biosynthesis-enabled 3D cellulose structures are highlighted. Their applications in tissue engineering, drug delivery, lightweight composites, thermal management, and energy storage are also discussed.



KEYWORDS: *bacterial cellulose, 3D printing, biocomposites, microbial biosynthesis, engineered living materials*

1. INTRODUCTION

The increasing emphasis and requirements on sustainability in today's world have led to a rapid shift from conventional polymers and composites to green and biobased materials.[1−3] Among those biobased polymers, cellulose is the world's most abundant, cost-effective, and easily accessible biopolymer, mostly commonly obtained from plants.[4,5] Cellulose can also be synthesized by certain types of bacteria during their metabolic processes, which is termed bacteria cellulose (BC).[6,7] BC has a combination of unique properties,[8,9] including high purity (lacking hemicellulose and lignin), high crystallinity, nanofibril network structure, high water retention, and high mechanical strength (tensile strength up to 2 GPa and Young's modulus of 138 GPa).[10,11] Moreover, the porous BC nanofiber network with high biocompatibility provides an ideal scaffold for tissue engineering.[12,13]

Since the discovery of cellulose-producing Acetobacter strains by Brown in the 1880s,[14] numerous aerobic bacterial species have been investigated and confirmed to generate extracellular cellulose. These include *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Komagataeibacter* (previously known as *Acetobacter* and *Gluconacetobacter*), *Pseudomonas*, and others.[15,16] Among these, *Komagataeibacter xylinus* is a particularly well-studied strain due to its ability to convert versatile types of organic substances to cellulose, not limited to glucose as the carbon source. The microbial biosynthesis of BC involves four key enzymatic steps:[17] (1) Small molecule precursor such as glucose is converted to glucose-6-phosphate using glucokinase as enzyme; (2) glucose-6-phosphate is isomerized to glucose-1-phosphate; (3) glucose-1-phosphate is converted to uridine diphosphate glucose by uridine diphosphate glucose pyrophosphorylase; and (4) cellulose synthase enzymes polymerize uridine diphosphate glucose into linear glucan chains. These chains undergo polymerization and crystallization into subfibrils, resulting in type I cellulose, which further aggregates into ribbon-like fibers of type II cellulose with diameters around 50 nm and lengths exceeding 100 μm.[15,18]

Despite its excellent physical properties in many aspects, BC has limited processability due to its high crystallinity, poor solubility, and incompatibility with many other synthetic materials.[19] The most common form of BC is 2D films or pellicles, which is because the cellulose-generating bacteria have high oxygen demand during metabolism,[20,21] so that the BC film is generally formed at air/liquid interface during cultivation. But for many practical applications, the BC

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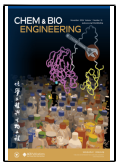

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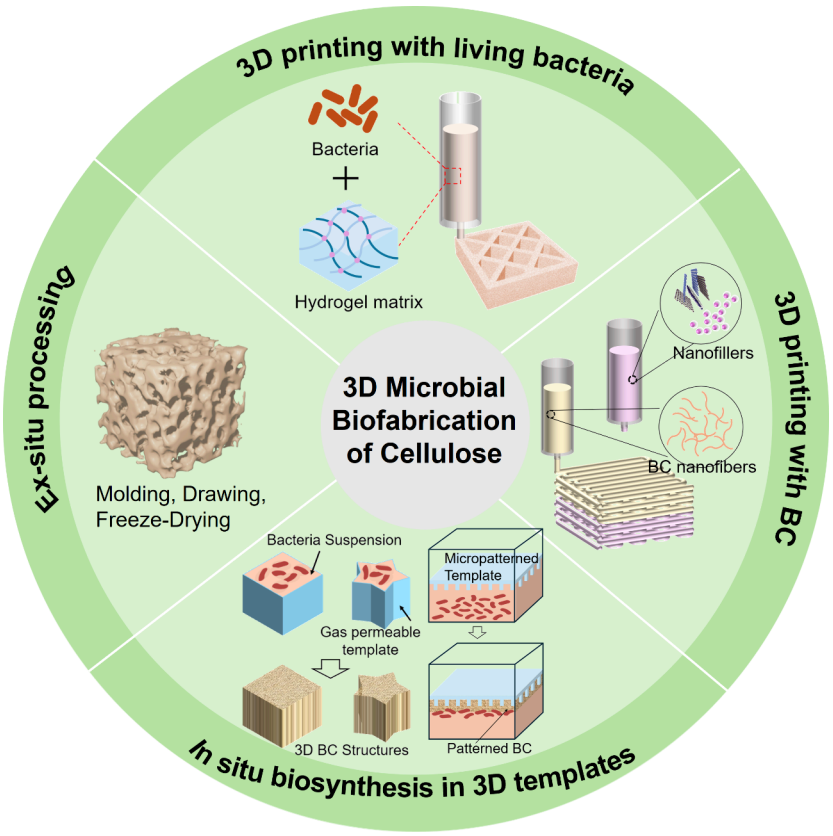

Figure 1. Overview of the main technologies for microbial biofabrication of 3D cellulose structures, which include ex situ processing, in situ biosynthesis in 3D templates, 3D printing with living cellulose-generating bacteria, and 3D printing with BC.

structures or devices need to have well-defined and customizable 3D geometry as well as tunable internal structure, which is very challenging to achieve by conventional liquid culture. Although it is possible to collect BC feedstock and then mold them into 3D shapes, such processes are not efficient and do not take advantage of the dynamic and living nature of microbial biosynthesis.[22]

Recent advancements in biofabrication especially 3D bioprinting provide new approaches that can potentially overcome the challenges in 3D BC structures fabrication.[23−25] The emerging concept of engineered living materials (ELMs) by integrating functional bacteria with synthetic materials also provides new possibilities in 3D BC fabrication.[26−29] In this Perspective, we conduct an overview and analysis of current technologies for fabricating 3D BC structures with a focus on those that utilize living bacteria and in situ biosynthesis (Figure 1). This can be achieved by two different methods: culturing cellulose-generating bacteria in 3D templates for in situ BC generation; or 3D printing with bacteria-containing living inks followed by BC cultivation. Those novel methods are critically evaluated and compared with conventional BC processing. We also discuss the recent applications of such 3D BC structures in tissue engineering, lightweight composites, thermal management, optics, and energy storage. The major challenges in this research area that need to be addressed are also critically examined.

2. MICROBIAL BIOFABRICATION OF 3D CELLULOSE STRUCTURES

Due to the unique physical properties of BC and its microbial biosynthesis-based production, the fabrication of 3D structures from BC is challenging and distinct from conventional polymers and composites. Here, we review the main technologies developed and organize them into three main categories (Figure 1). The first category is based on the conventional processing of BC as a biomaterial after it is biosynthesized, which is termed as ex situ processing. The second category is based on in situ BC biosynthesis by bacteria in 3D templates, which has high design freedom and tunability. The third category is based on 3D printing of living inks containing cellulose-generating bacteria and subsequent cultivation, this method integrates the advantages of 3D printing and microbial biosynthesis and has the potential to overcome limitations of conventional cellulose processing.

2.1. 3D BC by Ex Situ Processing. The utilization of BC as functional and sustainable biomaterials or for various applications has been explored for several decades. Conventional methods for BC processing are based on harvesting BC from their liquid culturing, followed by traditional polymer processing technologies. Such ex situ BC processing methods include stretching, molding, casting, fiber spinning, and freeze-drying.

BC films or pellicles from conventional microbial biosynthesis have a random network structure of nanofibers. Wang et al. showed that by wet-stretching of such BC films, high degree of alignment and dense packing of BC nanofibers can be achieved (Figure 2a,b).[30] Such anisotropic BC films show high strength and toughness, and they are also flexible and can be folded into desired shapes (Figure 2c). Bioinspired spiral hydrogel fibers were prepared from BC by wet-twisting,[31] and such BC hydrogel fibers show high strength and stretchability and can be potentially used for surgical sutures. Microbial

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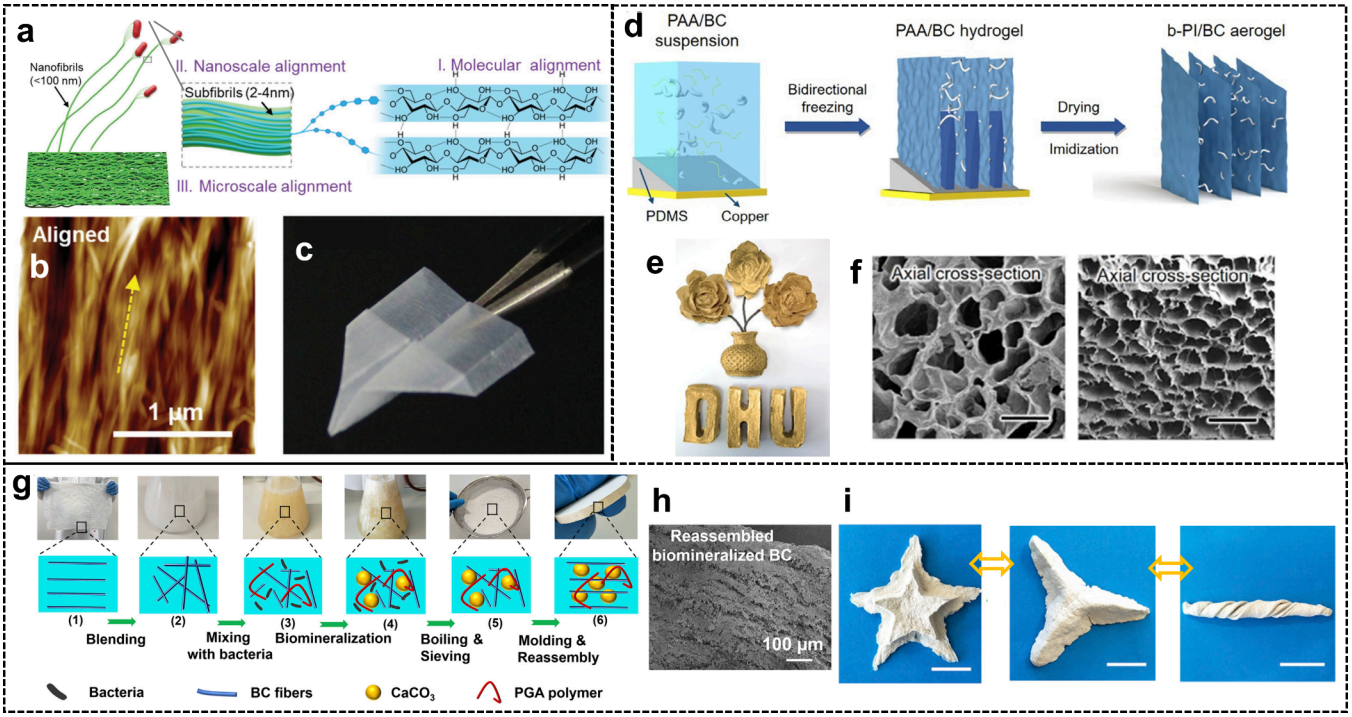

Figure 2. (a) Schematic of the formation of a strong and tough BC film by wet stretching. (b) AFM image of the aligned BC nanofibers. (c) Photo of an origami plane folded from the BC film. Reproduced with permission from ref 30. Copyright 2018, Wiley-VCH Verlag GmbH & Co. (d) Schematic illustration for the preparation of PI/BC aerogel through bidirectional freezing and subsequent imidization. (e) Photo of the PI/BC aerogels molded into various shapes. (f) Cross-section SEM images of PI/BC aerogels with random distributed (left) and unidirectional aligned (right) internal structures. Reproduced with permission from ref 36. Copyright 2020, Elsevier B.V. (g) Fabrication procedure of the biomineralized BC composites with all of the components obtained from microbial biosynthesis. (h) SEM image of the cross-section of biomineralized BC composite. (i) Various 3D objects molded from the biomineralized BC composites. Reproduced with permission from ref 38. Copyright 2021, The Authors, under the terms of the Creative Commons (CC BY 4.0) License https://creativecommons.org/licenses/by/4.0/.

biosynthesized BC pellicles can be converted into BC aerogel by directional freezing followed by freeze-drying,[32] which has good heat insulation and efficient sound adsorption.

To enhance the processability of BC and achieve synergistic properties, other types of synthetic or natural polymers are often used in combination with BC during fabrication. For instance, BC was used to fabricate double-network hydrogels with chemically cross-linked polyacrylamide as the first network and borax cross-linked BC nanofibers as the second network.[33] Such hydrogels showed very high stretchability and can be used as flexible sensors due to their high ionic conductivity. In another report,[10] by coating the dried BC film with alginate and then rolling it up into hollow tubes, strong and biodegradable straws can be obtained, which have better strength and performance than paper straws. Lightweight and strong hierarchical composites were also demonstrated by in situ fermentation with polyvinyl alcohol (PVA) followed by freezing-thawing.[34] Sulfonated BC and gelatin were combined to fabricate biocompatible scaffolds for hepatocytes.[35] Such biopolymer scaffolds showed high porosity, good cytocompatibility, and hemocompatibility and can be potentially used for liver tissue engineering. Aerogels with anisotropic structures and properties were fabricated by combining polyimide (PI) and BC and subsequent bidirectional freezing (Figure 2d,e).[36] Such PI-BC aerogels showed well-defined and tunable porous internal structures (Figure 2f). Low thermal conductivity with very different values along the in-plane and through-plane directions was obtained. BC/potato starch biocomposite was prepared through in situ biosynthesis and freeze-drying, followed by processing into a tubular shape.[37] The vascular-shaped composites demonstrated 75% patency and rapid blood vessel regeneration through in vivo testing.

However, BC can also be integrated with inorganic materials for the fabrication of 3D organic−inorganic biocomposites with enhanced physical properties. For instance, Yu et al.[38] showed that by combining three different components from microbial biosynthesis: BC, $CaCO_3$, and poly(γ-glutamic acid) (PGA), sustainable biocomposites with tunable internal structure and mechanical properties can be obtained (Figure 2g,h) Such biomineralized BC with high toughness and impact resistance can be molded into any desired 3D geometries and repeatedly recycled (Figure 2i). Porous BC structures with aligned nanofibers were also used for mineralization to introduce hydroxyapatite (HAP) nanoparticles inside the BC scaffold.[39] Such BC-HAP composites showed a relatively high modulus and hardness. Similarly, BC-alginate hydrogel was reinforced with MXene and studied for bone tissue engineering application.[40] Such BC composite hydrogel showed high porosity and enhanced osteogenic effect at a low content of MXene. BC was mixed with graphene oxide (GO) and such mixture was used for aerogel fabrication followed by chemical or thermal reduction of GO.[41] The obtained BC/rGO aerogels had high thermal stability and electrical conductivity. In another report,[42] BC was blended with alginate and then cast and freeze-dried to form a BC-alginate foam, such foam can also be further carbonized to achieve high electrical conductivity and low density. Such BC-graphene aerogels

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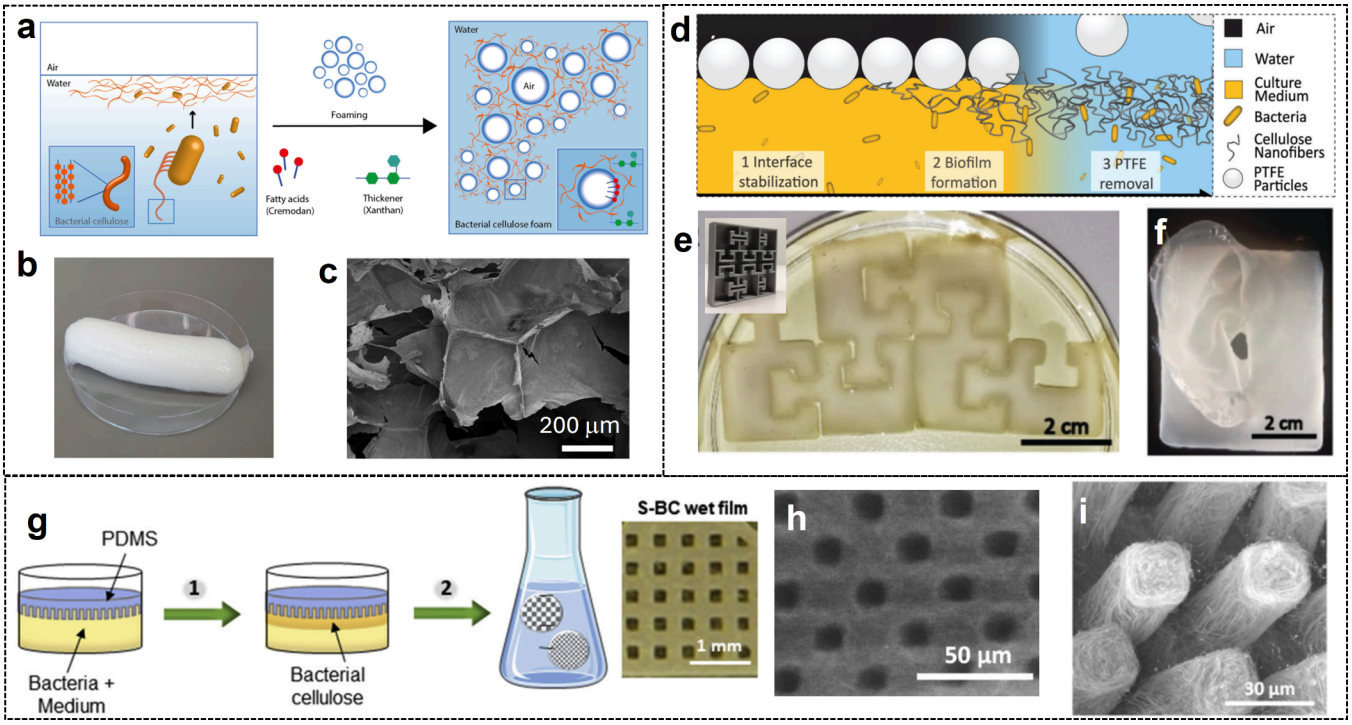

Figure 3. (a) Schematic of the BC foam formation process by in situ cultivating. (b) Photo and (c) cross-section SEM of a generated BC foam. Reproduced with permission from ref 44. Copyright 2018, The Authors, under the terms of the Creative Commons (CC BY 4.0) License https://creativecommons.org/licenses/by/4.0/. (d) Schematic of the self-assembly process of PTFE particles enabling 3D biofabrication of nanocellulosic materials. (e) Puzzled-shaped BC objects fabricated by this method, and the inset is the puzzle mold. (f) Hollow ear-shaped BC produced by using a superhydrophobic 3D mold. Reproduced with permission from ref 45. Copyright 2018, Royal Society of Chemistry. (g) Schematic of biolithography process. The patterned BC gels were fabricated by in situ fermentation with a patterned PDMS film on top. (h) SEM images of an array of patterned holes in BC with 20 um diameter. (i) SEM image of an array of columns of BC with 20 um size. Reproduced with permission from ref 47. Copyright 2021, Elsevier B.V.

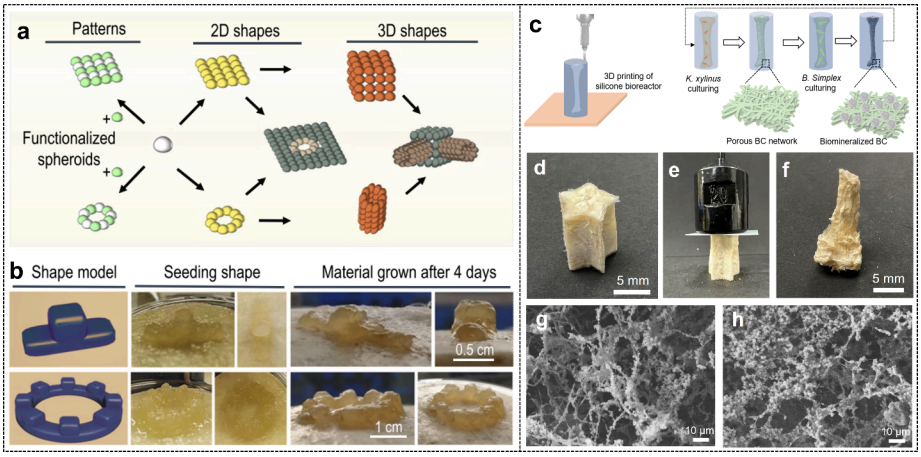

Figure 4. (a) Schematic of potential 2D and 3D structures that can be built from BC spheroids. (b) Growth of two example 3D shapes constructed using BC spheroids Model of desired structure (left), the seeded spheroid shape assembled manually (middle), and the resultant material structure produced after 4 days of further growth (right). Reproduced with permission from ref 50. Copyright 2021, The Authors, under the terms of the Creative Commons (CC BY 4.0) License https://creativecommons.org/licenses/by/4.0/. (c) Schematics of the programmable microbial synthesized method for 3D BC biocomposites. *K. xylinus* and *B. simplex* bacteria were sequentially cultured in a 3D-printed gas-permeable bioreactor. (d,e) BC-$CaCO_3$ biocomposite with star cylinder shape and demonstration of its strength by supporting a 20 g standard weight. (f) Bone-shaped BC-$CaCO_3$ biocomposite from microbial biosynthesis. (g,h) Cross-section SEM of the BC-$CaCO_3$ biocomposites prepared by (g) one-cycle biosynthesis and (h) two-cycle biosynthesis. Reproduced with permission from ref 51. Copyright 2024, American Chemical Society.

have also been used for wireless flexible pressure and humidity sensors with high sensitivity and cycling stability.[43]

2.2. 3D BC by In Situ Culturing in 3D Templates/Reactors. Despite the convenience of ex situ processing of BC, those methods are largely based on conventional polymer processing and do not take advantage of the flexibility and living nature of microbial biosynthesis. Conventional microbial biosynthesis of BC from liquid culturing can only generate 2D film at air/liquid interface; to address this limitation, several approaches have been explored. The first approach is based on

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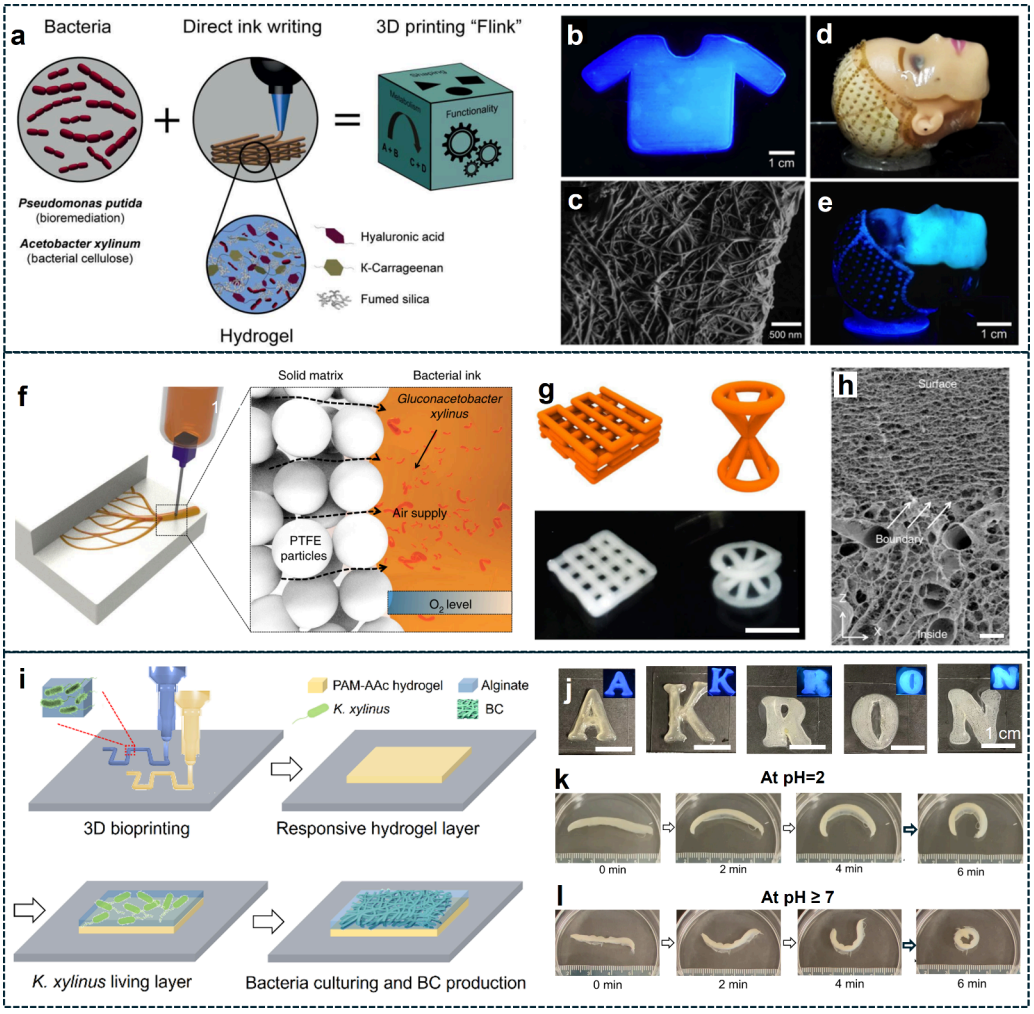

Figure 5. (a) Schematic of 3D printing of living inks for the creation of functional living structures. (b) Fluorescent image of a T-shirt shaped 3D-printed BC structure. (c) SEM image of BC generated in a 3D-printed living structure. (d,e) 3D-printing with living ink on the surface of a doll's face and subsequent formation of a cellulose-reinforced hydrogel conformal to the surface. Reproduced with permission from ref 54. Copyright 2017, The Authors, under the terms of the Creative Commons (CC BY 4.0) License https://creativecommons.org/licenses/by/4.0/. (f) Schematic of DIW 3D printing with bacteria-laden ink into a supporting granular gel. (g) After cultivation, the formed 3D BC structure can be taken out without damage. (h) Cross-sectional SEM image of the generated BC structures. Reproduced with permission from ref 55. Copyright 2019, The Authors, under the terms of the Creative Commons (CC BY 4.0) License https://creativecommons.org/licenses/by/4.0/. (i) Scheme of the fabrication of bifunctional living gel structures with shape changing capability, which is based on DIW 3D printing with stimuli-responsive hydrogel and *K. xylinus* embedded living gel. (j) Photos of the 3D printed living gel structures after in situ BC generation. (k,l) Photos of self-folding process of the bifunctional living gel at acidic and basic pH conditions, respectively. Reproduced with permission from ref 57. Copyright 2024, American Chemical Society.

the foaming of a bacterial liquid suspension so that 3D porous BC structures can be obtained after biosynthesis in the foam template. For instance, Ruhs et al.[44] reported a method for the fabrication of microporous BC foam by foaming the *G. xylinus* liquid suspension with the presence of fatty acid as a surfactant and Xanthan as the thickener (Figure 3a). A stable BC foam structure can be obtained after 4 days (Figure 3b,c).

The second approach is based on culturing cellulose-generating bacteria in a gas-permeable hydrophobic template, and the obtained BC structures conform to the overall geometry of the template. For instance, Greca et al.[45] described a simple and customizable biofabrication process to achieve hollow and seamless 3D hollow BC structures using hydrophobic polytetrafluoroethylene (PTFE) particles and superhydrophobic molds (Figure 3d–f). The resulting 3D BC structures showed good mechanical properties, chemical resistance, and biocompatibility to be used as advanced materials in regenerative medicine applications. Building on this method, the same group successfully engineered pH-responsive, nontoxic 3D BC microcapsules for colon-targeted multidrug delivery in oral therapy.[46]

Besides the macroscopic shape and geometry control of the 3D BC structures discussed above, lithography-based patterning can be used in combination with bacteria culturing to achieve well-defined BC patterning with resolution on the micrometer scale. For instance, Riog-Sanchez et al.[47] showed that by placing a soft micropatterned polydimethylsiloxane (PDMS) mold on top of the liquid medium of *K. xylinus*, the generated BC pellicle had the corresponding patterns on its bottom surface (Figure 3g–i), and this method was termed biolithography. Such micropatterned BC structures have promising applications in microfluidics, optics, and photonics. In another report,[48] by placing the liquid mixture of *K. xylinus* suspension and agarose in a 3D PDMS mold and controlling

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the oxygen inflow, intricate 3D BC structures with detailed replicas of human features, such as a doll's face and ear, were successfully obtained. Micropatterned BC structures have also been demonstrated by culturing *K. xylinus* on micropatterned templates fabricated by two-photon polymerization.[49]

The third method is based on the controlled assembly of bacteria-containing spheroids, and 2D/3D BC patterns can be obtained after culturing the living spheroids for several days. For instance, Astorga et al.[50] developed an approach for making spheroids containing genetically engineered *K. rhaeticus*, and used the living spheroids as the building blocks for fabricating patterned ELMs (Figure 4a). Various 2D and 3D structures (Figure 4b) were fabricated from them after seeding and culturing. Moreover, they showed that such a BC spheroid-based ELM can respond to specific chemical signals by designing a simple barcode composed of patterned spheroids with either wild-type bacteria or pReceiver bacteria.

Beyond the fabrication of 3D BC structures from those in situ templating methods, 3D biocomposites with a BC matrix can also be fabricated with a similar approach. By incorporating and culturing multiple types of functional bacteria in 3D-printed bioreactors, the controlled biosynthesis of both BC and inorganic materials within the same 3D structures can be potentially achieved. Our group recently demonstrated the fabrication of 3D hierarchical bone-mimetic biocomposites based on this approach (Figure 4c).[51] Specifically, we first fabricated 3D soft and gas-permeable bioreactors by extrusion-based 3D printing of silicone elastomer. Then, liquid suspensions of *K. xylinus and B. simplex* P6A bacteria were sequentially loaded into the bioreactor. BC and $CaCO_3$ particles were generated by those two types of bacteria within the 3D bioreactor, respectively. After several cycles of culturing, BC-$CaCO_3$ biocomposites with precisely controlled shapes and enhanced mechanical properties can be obtained (Figure 4d–f). Such biocomposites have highly tunable porosity and internal structure (Figure 4g,h), and they can further be transformed into BC-CaHA (calcium hydroxyapatite) biocomposites after chemical conversion. This work represents a new and versatile way of 3D biocomposite fabrication with promise in bone tissue engineering.

2.3. 3D BC by 3D Printing. Additive manufacturing or 3D printing of polymers has experienced tremendous progress in the past decade or so, and multiple types of polymer 3D printing technologies including material extrusion-based, vat polymerization-based, and powder bed fusion-based methods have been developed. BC as a type of biopolymer with unique properties has also been explored as a feedstock or nanofiller in 3D printing. More importantly, 3D printing can be used to directly fabricate 3D living structures containing cellulose-producing bacteria, and well-defined 3D BC structures can be obtained after culturing.

In order to be compatible with living bacteria, the 3D printing process needs to be conducted under ambient conditions with sufficient access to water and nutrients for the bacteria. Therefore, direct ink writing (DIW) is the method of choice. DIW is based on the extrusion of soft inks by pressure or a piston in a layer-by-layer manner onto the building platform. Hydrogel is the most commonly used soft ink for DIW, and its high water content is important to maintain the viability of biological cells embedded in it. Such soft inks also need to have shear-thinning and thixotropic properties to be successfully printed and maintain the structural integrity.

2.3.1. 3D BC by 3D Printing of Cellulose-Generating Bacteria. DIW 3D printing of ELMs has attracted increasing attention due to the potential to fabricate 3D living structures. For instance, cyanobacteria were incorporated in a gel matrix and 3D printed into well-defined 3D shapes,[52] those printed ELMs depend on only light energy and CO_2 as a carbon source for growth, and can be used in bioremediation. Genetically engineered *E. coli* has also been embedded in alginate and 3D printed onto calcium-containing substrates,[53] and patterned biofilm with curli fiber formation was demonstrated.

DIW printing of cellulose-generating bacteria containing living ink is a promising approach for the customizable fabrication of 3D BC structures. This method has the potential to overcome the limitations of conventional liquid culturing by enabling complex 3D geometry fabrication. For instance, Schaffner et al.[54] showed that by embedding *K. xylinus* in a hydrogel matrix (composed of hyaluronic acid, k-carrageenan, and fumed silica), 3D-printable living ink can be obtained (Figure 5a). DIW 3D printing with such living ink enabled the fabrication of patterned BC structures (Figure 5b,c). BC generation in the 3D-printed hydrogel depended on the oxygen availability and matrix viscosity. The printing can also be conducted on a curved template for tissue engineering applications (Figure 5d,e).

In another report,[55] by 3D printing of *K. xylinus* containing hydrogel inside a gas-permeable PTFE microparticle solid matrix, 3D BC structures can be generated after cultivation (Figure 5f). They also showed that the mechanical strength of such BC structures can be improved by ionic cross-linking. Tubular BC structures were also fabricated that can potentially be used for vascular tissue engineering (Figure 5g,h). A similar approach by DIW 3D printing of *K. xylinus* liquid suspension inside a silicone-based granular gel was also reported.[56] 3D BC structures were generated after culturing due to the gas permeability of the granular gel matrix. The structures also showed self-healing properties due to the existence of living bacteria.

Beyond those 3D BC structures produced by DIW 3D printing and in situ BC generation, dynamic 3D BC structures with shape-changing capability can have important applications in soft actuators or soft robotics. We recently demonstrated that by 4D printing with living inks containing *K. xylinus*, shape transformation of in situ generated BC structures can be achieved upon pH changes (Figure 5i).[57] This method was based on DIW printing of *K. xylinus*-embedded living hydrogel ink on top of a pH-responsive hydrogel, and when the responsive hydrogel layer swells or shrinks upon pH changes, the internal strain mismatch leads to overall bending or folding in a reversible way (Figure 5j–l).

2.3.2. 3D Printing with BC-Containing Ink. Due to its unique nanofiber morphology, high crystallinity, excellent biocompatibility, and mechanical strength, BC has also been used as the main component or additive in DIW 3D printing inks. Many such studies are targeted toward biomedical applications, especially tissue engineering. BC needs to be chemically modified in some cases in order to enhance compatibility with other materials and achieve high printability. For instance, BC was surface-modified by a carboxymethylation process to introduce negative charges,[58] and then blended with positively charged chitosan. Such biopolymer mixtures showed good compatibility with microvessel endothelial cells and was used in DIW 3D printing for tissue engineering. In another report, BC was oxidized using 2,2,6,6-tetramethylpi-

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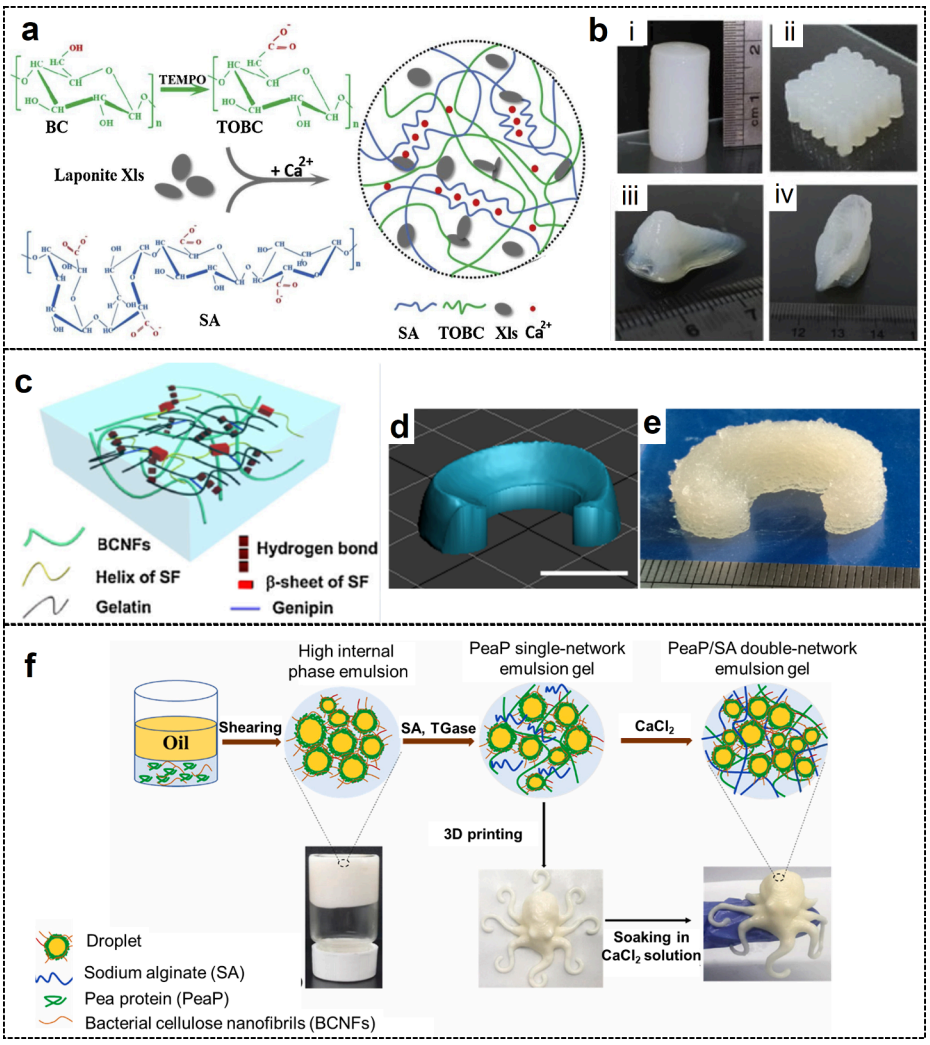

Figure 6. (a) Chemical composition of the 3D-printable hydrogel ink containing TEMPO-modified BC, laponite, and sodium alginate, and further ionically cross-linked by Ca^{2+}. (b) Photos of various 3D-printed structures including cylinder, cubic, human nose, and human ear. Reproduced with permission from ref 59. Copyright 2020, Elsevier B. V. (c) Schematic of the hydrogel network composed of BC, gelation, and silk fibroin. (d,e) 3D model of human meniscus and the printed structure; scale bar is 10 mm. Reproduced with permission from ref 61. Copyright 2019, Elsevier B. V. (f) Schematic of the formation of 3D-printable double-network emulsion gels containing BC, pea protein, and alginate. Photo of the gel ink and a 3D-printed jellyfish structure is also shown. Reproduced with permission from ref 69. Copyright 2023, Elsevier B. V.

peridinyl-1-oxyl (TEMPO) and subsequently mixed with alginate and nanoclay to formulate a 3D printing ink (Figure 6a).[59] The 3D-printed structures showed structural stability in a physiological environment (Figure 6b) and also enabled a continuous release of protein, indicating potential for drug release and tissue engineering applications. BC was chemically modified with TEMPO and maleic acid followed by mixing with gelatin, and then used for DIW 3D printing into 3D scaffolds with good osteoblast viability for bone regeneration.[60]

There are several other studies using a similar strategy, and some representative examples are summarized here. BC was combined with silk fibroin and gelatin for DIW 3D printing of 3D scaffolds with tunable porosity and high mechanical strength (Figure 6c–e).[61] Those 3D scaffolds were also implanted under the dorsal skin of mice to demonstrate their good biocompatibility. Surface modified BC was used as the cross-linkers and nanofillers in a UV-curable ink for DIW 3D printing with enhanced mechanical properties.[62] BC was incorporated into gelatin methacryloyl hydrogel for 3D printing and the fabricated 3D structures were used for cartilage tissue engineering with promising results.[63] BC was used in combination with pea protein and alginate to form a 3D printable double-network gel (Figure 6f), and such high internal phase emulsion gels showed enhanced viscoelasticity, fracture stress, and thermal stability, offering a promising approach for developing plant-based meat products.

BC was used as the rheological modifier and binder together with polyamic acid for DIW 3D printing,[64] and the printed structures were converted into polyimide/BC aerogel after freeze-drying and thermal imidization. Such a 3D polyimide/BC aerogel with highly porous internal structure showed lower thermal conductivity and enhanced mechanical properties for thermal insulation applications. In addition to DIW 3D printing, BC can be micronized and blended with thermoplastic to produce composite filaments for fused deposition modeling (FDM) 3D printing.[65] The resulting 3D-printed scaffolds demonstrated low cytotoxicity and good cell adhesion.

For many functional applications, such as bone tissue engineering and electronics, it is necessary to integrate BC with

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inorganic structures during the 3D fabrication process. For instance, 3D printable composite hydrogels composed of BC, alginate, and in situ generated copper nanoparticles have been developed and used for DIW 3D printing.[66] Such composite hydrogels showed antimicrobial properties with application in wound dressing. In another report, BC, poly(vinyl alcohol), and hexagonal boron nitride were combined into DIW 3D printing inks and studied as bone tissue engineering scaffolds.[67] Similarly, composite hydrogels composed of BC, polycaprolactone, gelatin, and hydroxyapatite were used for DIW 3D printing for bone tissue engineering.[68] Good biocompatibility, mechanical strength, and high porosity were achieved in these 3D composite structures.

3. APPLICATIONS

Due to the excellent biocompatibility and biostability of BC, its 3D structures have been extensively studied for biomedical applications, especially tissue engineering. Tubular BC was studied as vascular grafts,[70] which showed higher tensile strength than native vessels and the luminal surface showed a similar porous structure as native vessels. In vivo study showed those BC grafts had over one month patency, neovessels, and endothelial cells were present on the luminal surface of the graft. BC hydrogels with embedded Au NPs were also used for bone tissue engineering study.[71] The controlled release of Au NPs from such porous BC hydrogel was shown to be able to facilitate osteogenic differentiation of human bone-marrow-derived mesenchymal stem cells. Highly porous 3D BC sponges were fabricated for tissue engineering applications. For instance, Frone et al.[72] partially replaced the −OH groups in BC with amino groups and cross-linked the BC network using eco-friendly, nontoxic cross-linkers. In vivo tests indicated that the BC sponge is noncytotoxic and does not cause any inflammatory response in macrophages. Similarly, Xun et al.[73] cross-linked the BC network with a nontoxic cross-linker to form macroporous BC scaffolds with excellent compression and recovery abilities. These 3D BC scaffolds successfully demonstrated neocartilage tissue regeneration in an in vivo test. BC-based structures with hollow microchannels have also been used for vascularized breast tumor model.[74]

Porous and lightweight BC aerogels also hold great potential for molecular separation, such as oil−water separation. These BC aerogels are prepared by freeze-drying with tunable porosity and high compressibility.[75] Surface modification and carbonization can also be applied to BC aerogels to impart hydrophobic properties and improve oil absorption capability. For instance, surface-modified 3D BC aerogel with a high water contact angle of 142°, excellent oil absorption capacity (121.8−284.1 g/g) and a 96.7% efficiency in organic solvent−water separation was demonstrated.[76] In another report, BC aerogel with subsequent carbonization led to 99.5% compressibility and an oil absorption capacity of 132−274 g/g.[77] Similarly, carbonized BC was incorporated in PDMS sponge to create a superhydrophobic aerogel with a 146° water contact angle,[78] which maintained a superstable oil absorption efficiency of 99.3% even after 30 compression cycles.

Moreover, due to its highly porous structure and low thermal conductivity, BC aerogel has been explored for thermal insulation applications. For instance, a hydrophobic BC/PVA bilayer aerogel was fabricated and showed effective thermal dissipation due to its high visible reflectance and strong emissivity.[79] Flame-retardant and heat-insulating BC-zinc borate composite aerogel was also reported.[80] In another report, BC aerogel was modified by silylation and showed superelasticity, high flexibility, low thermal conductivity (27.3−29.2 mW/mK) and high heat shielding efficiency, along with exceptional selective oil absorption capacity and recoverability.[81]

Micropatterned BC composite structures have also promising applications in optics, electronics, and energy storage applications.[82,83] For instance, BC-SiO_2 composites with intricate patterns of ∼10 μm precision have been demonstrated as nonlocal holographic metalens and 3D imaging metalens, realizing complicated acoustic holograms and high-resolution 3D ultrasound imaging in far fields.[84] BC has also been integrated with functional inorganic nanomaterials and used as electrolytes or electrode precursors for energy storage devices.[85] The presence of BC enhances the loading capacity of active materials and leads to hierarchical porous structure. For instance, zinc ion batteries with BC hydrogel electrolyte and BC composite-derived electrode were reported with Coulombic efficiency reaches >99% with 88.2% capacity retention after 1000 cycles.[86]

4. CONCLUSIONS AND OUTLOOK

In summary, in this Perspective, we summarized the recent progress in the creation of functional 3D BC structures with a focus on the different types of fabrication technologies and potential applications. In terms of fabrication technologies, innovative in situ cultivation in 3D templates and 3D printing of bacteria-embedded living inks provide more flexibility and functionalities than conventional polymer processing. Such 3D BC and composite structures with well-defined geometry, composition, and internal structures have promising applications in tissue engineering, thermal management, molecular separation, flexible electronics, and energy storage.

However, there are also several important challenges that need to be addressed before the full potential of functional 3D BC structures and devices can be achieved. In terms of BC's microbial synthesis and its intrinsic properties, the challenges are summarized here. First, there are limited selections of fermentation substrates for BC production, and the commonly used ones (glucose, sucrose, and mannitol) have relatively high cost.[87] Second, contamination by other microorganisms during fermentation is possible, and as-biosynthesized BC needs to be extensively purified to remove bacteria residual for many applications. Third, BC has very limited biodegradability under physiological conditions, which constrains its wider applications in tissue engineering. This can be potentially addressed by incorporating cellulase enzyme or genetic modification of cellulose-generating bacteria.

There are also major challenges with regard to 3D fabrication with BC and their applications. First, the biosynthesis of materials by native microorganisms is relatively slow, so large 3D structures take days or weeks to complete by the in situ method. But with the rapid development of genetic engineering, microbial biosynthesis processes can be accelerated. One recent report showed that by using high throughput droplet-based microfluidic platform to screen and analyze cellulose-producing bacteria *K. xylinus*,[88] faster identification of genotype−phenotype links can be achieved to accelerate BC production. Second, achieving a high degree alignment of BC nanofibers in macroscopic 3D structures is still challenging. The random motion or orientation of cellulose-producing bacteria in liquid or hydrogels is hard to control. Such an alignment of BC nanofibers is important to

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achieve superior mechanical strength, precise porosity, and rapid water/ion transport in many applications. Potential approaches to align BC nanofibers include using wet stretching, magnetic field, and nanopattern templating. Third, integrating cellulose-producing bacteria with functional devices and synthetic structures while maintaining their bioactivity is difficult to achieve. The temperature, water, and nutrient access requirements are not compatible with many electronic and energy devices. The potential solutions for such a challenge can be from advanced electronic packaging, microfluidics, and droplet-based fabrication. Last but not least, there are environmental and regulatory concerns with the use of 3D living structures, which include the potential environmental impact of releasing engineered bacteria from them and the absence of clear regulatory guideline for living materials.[89] Those challenges and opportunities are critical for the future development of this exciting area that integrates microbial biosynthesis with advanced manufacturing.

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Notes

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