OVERVIEW OF INEXPENSIVE PRODUCTION ROUTES OF BACTERIAL CELLULOSE AND ITS APPLICATIONS IN BIOMEDICAL ENGINEERING

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Bacterial cellulose (BC) is an innovative polymeric nanofibre, which meets the demands of rapidly advancing industries, such as biomedical engineering, food packaging, pulp and paper and electrical appliance engineering. The versatility of bacterial cellulose is largely due to its unique properties, such as high crystallinity, high thermal stability, high water absorption capacity, hydrophilicity, good mechanical strength, biodegradability, high biocompatibility and high porosity, making it well-suited for applications in various fields. In recent years, advances for enhancing the applicability of BC through modification and its inclusion into composites have been in focus. Unfortunately, despite the multiple advantages it offers, the production cost of BC is too high, thus reducing/limiting its commercial attractiveness and industrial scale production. This paper is an overview of the current research trends for developing cheaper BC production pathways and of recent advances performed so far with the prospect of enhancing its potential application in biomedical engineering.

Keywords: bacterial cellulose, nanofibre, biomedical engineering, composites

INTRODUCTION

Cellulose is one of the most abundant biopolymers synthesized by many plants and microorganisms.[1] It has been estimated that about 10^{12} tonnes of this biopolymer are produced each year, thus there is a continuous supply of renewable and biodegradable raw material. Natural cellulose from plant sources has been widely used as raw material in various manufacturing sectors, such as textiles, and pulp and paper based products.[2] Biopolymers of plant and bacterial origin have long been in use for biomedical application.[3] However, the massive use of plant cellulose has taken a heavy toll on the environment. Plant cellulose is intertwined with hemicellulose and lignin, which are considered as unwanted impurities, thus additional processing is required to obtain pure cellulose. In order to remove these components, plant cellulose undergoes a harsh chemical separation process, using alkaline and acid treatments.[4,5]

Over the last decade, research on the production of cellulose using microorganisms has been intensively conducted with the aim of providing an alternative for plant cellulose.[6,7] Bacterial cellulose (BC) exhibits higher purity, compared to plant cellulose, as it contains neither

hemicellulose nor lignin. Moreover, a small amount of time is needed to synthesize BC, compared to plant cellulose, which takes a longer period to grow and mature. These features make BC an attractive material for a wide range of applications, including biomedicals, food technology, packaging industry, pulp and paper industry, as well as in engineering of electrical appliances. Therefore, BC is considered as one of the most important and innovative materials for rapidly advancing industries. This paper reviews the current research undertaken on BC production and the developments achieved with the prospect of its multiple potential applications in the field of biomedical engineering.

BIOSYNTHESIS, STRUCTURE AND PROPERTIES OF BC

BC is synthesized *via* the oxidative carbohydrate metabolic activity, which is carried out through an enzymatic process involving four different enzymes, *i.e.* glucokinase, phosphoglucomutase, uridinediphosphate (UDP)-glucose pyrophosphorylase and cellulose synthase (Fig. 1).[8] The conversion of carbohydrate into BC involves four stages of intermediate synthesis: (1) the initial process is triggered by the phosphorylation of glucose monomers to generate glucose-6-phosphate (G6P) intermediates by glucokinase;[9] (2) the G6P is then converted into glucose-1-phosphate (G1P) by phosphoglucomutase; (3) then, UDP-glucose, an important precursor for cellulose assembly, is synthesized from G1P by UDP-glucose pyrophosphorylase; (4) finally, after BC precursors are synthesized, these units assemble and translocate across the inner membrane by a complex of cellulose synthase subunits.[9]

During cellulose synthesis, BC monomer units, which are produced in the interior of the cell, are spun out from the transporter nozzles and form units known as protofibrils. These protofibrils assemble to form a structure known as ribbon of cellulose nanofibrils. These ribbons construct a web-shaped network, which gives BC its three-dimensional structure. The three-dimensional fibrous network of BC also provides its porous matrix.[10]

Understanding the structure of cellulose is of foremost importance to be initially ensured, before exploiting this renewable resource for various technological applications, particularly considering that the structure of cellulose differs in terms of size, depending on its origin. Therefore, to describe the structure of this biopolymer, it is necessary to first elucidate its synthesis process as a key to addressing BC nanoconstruction.

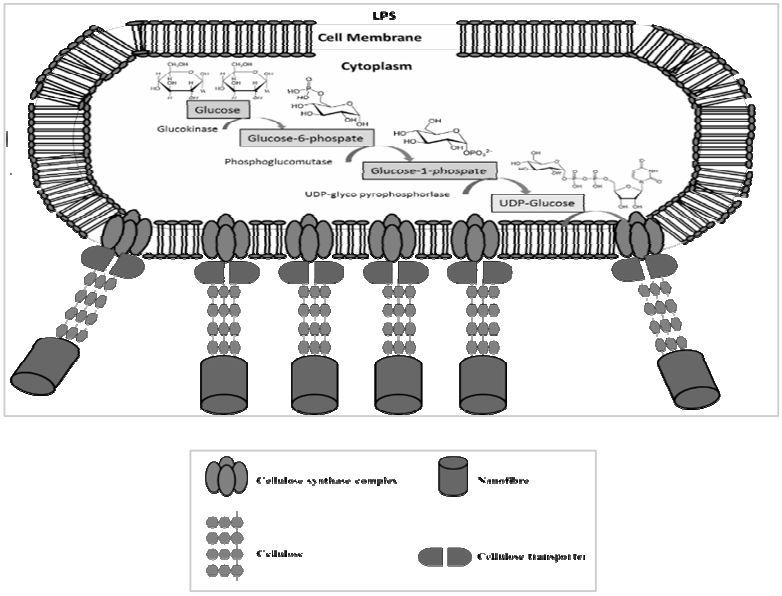

Figure 1: BC biosynthesis occurring in single-celled microorganisms, such as *G. xylinus*

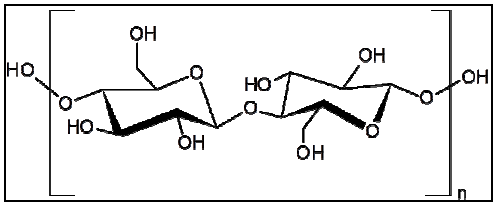

Figure 2: Chemical structure of a cellobiose unit

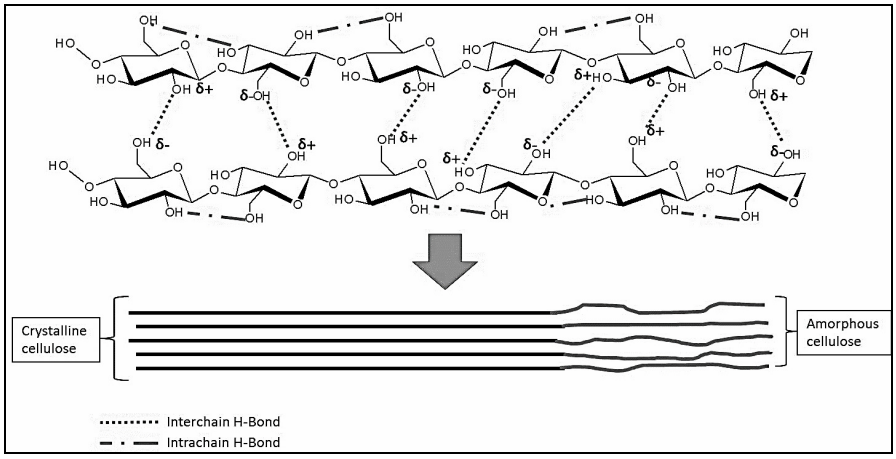

Figure 3: Polymerization of glucan chains and formation of crystalline and amorphous cellulose

Cellulose is a linear polymer composed of D-anhydro-glucopyranose ($C_6H_{11}O_5$) monosaccharide, which is an aldehyde sugar.[11] This single unit takes on either α-glucose or β-glucose, depending on the position on the hydroxyl group. When this unit forms disaccharides, they are known as cellobiose units (Fig. 2). Cellobiose is a reducing sugar, comprising two β-glucose molecules that are linked by a β-1-4 glucan chain.[6]

BC nanoconstruction can be divided into two main steps. The first consists of the polymerization of saccharide monomers joined *via* glucan chains. The linkage of the monomers is stabilised by hydrogen bonding of the intrachains between the hydroxyl groups and oxygen of the adjacent ring and form a linear configuration chain. Then follows the post-polymerization step, in which individual glucan chains combine and form crystalline cellulose.[8,12] Parallel stacking of multiple cellulose chains forms elementary fibrils, which further aggregate to construct larger microfibrils with diameters ranging between 5 and 50 nm. The lateral arrangement of microfibrils, as a function of the length, number and organization of the glucan chain in BC is highly dependent on the formation of intra- and inter-hydrogen bonding between neighbouring molecules. BC comprises a mixture of crystalline and amorphous structure domains,[11,13] while the hydrogen bonding network makes it a relatively stable biopolymer[14] (Fig. 3).

Several parameters need to be considered in evaluating BC for various applications, including its water holding capacity, mechanical strength and biocompatibility, and it is also noteworthy that the remarkable properties of BC are greatly influenced by its nanoconstruction.

Crystallinity is an indicator of cellulose purity,[15] most of the cellulose synthesized by microorganisms are much purer compared to plant cellulose, possibly because they do not contain lignin and hemicellulose as in plant cellulose.

Mechanical strength is also an important feature when considering possible BC applications; it is determined by the degree of polymerization (DP) and the lateral arrangement of the microfibrils. The DP represents the number of monomer units involved in the BC biopolymer nanoconstruction, which correlates with the mechanical strength of the BC nanofibrils. The DP value of BC varies based on its source and ranges between 2,000 and 8,000.[13] A high DP will provide superior tensile strength and high Young's modulus (elasticity). The arrangement of

cellulose chains in a parallel manner creates sheets of BC, which are stabilised by weak hydrogen bonds (both intra- and intermolecular) and van de Waals forces. These H-bonds and forces make BC hydrophilic and able to retain water molecules within its pores.

Water holding capacity (WHC) is the ability of BC to retain a certain amount of water or solution. WHC is an important parameter, whereby high WHC is suitable for biomedical application, especially in wound healing. The capacity of BC to have a high or low value of WHC depends on the porosity and the surface area formed during BC nanoconstruction. The increase in pore size and surface area will increase the amount of water that can penetrate and be trapped within the BC matrix.[16] However, there is no standardized procedure available to measure the water holding capacity of BC, which makes it challenging to compare different findings.

LOW-COST PRODUCTION OF BC

BC is naturally produced by some species of bacteria, such as *Acetobacter xylinum*, *Agrobacterium*, *Gluconacetobacter*, *Rhizobium*, *Achromobacter*, *Alcaligenes*, *Aerobacter*, *Azotobacter*, *Salmonella*, *Escherichia* and *Sarcina*.[1,5,17-19] The cellulose nanofibres produced by these microorganisms have attracted the researchers' attention due to their exceptional characteristics, as well as their eco-friendliness. *G. xylinus* has been commonly investigated for BC production. This is due to the ability of its strains to efficiently synthesize BC from various carbon sources, as well as provide a high yield in BC production.[20,21] Both synthetic and non-synthetic culture media can be used to produce BC through the oxidative fermentation process. In BC synthesis, any form of carbon source will be converted to G6P before the enzymatic process to produce cellulose. The biggest advantage of using bacteria as a cellulose producer lies in their ability to adapt and utilize various sources of carbon to synthesize cellulose.[22] Other unique features of bacteria as cellulose producers, compared to their plant counterpart, are their high cellulose production rate and their feasibility in regulating cellulose production.

Although BC has very interesting features, fuelling its choice as an alternative for plant cellulose, limitations and challenges in BC production still remain.[7] The production cost of BC is too high, thus making its production inefficient and the product less commercially attractive, as well as limiting the industrial scale production.[4] Three different optimized concentration media known to support the growth of *G. xylinus* were supplemented with five different carbon sources (date syrup, food-grade sucrose, glucose, mannitol and sucrose) and tested for BC production by Muhammadkazemi *et al*.[23] In their study, they found food-grade sucrose and date syrup to be unsuitable carbon sources in growing *G. xylinus* to produce BC, but mannitol and sucrose yielded better results in two of the three media used. They also highlighted that improved thermal stability and crystallinity were observed when one of the media was supplemented with mannitol and this might be due to the nitrogen source as suggested. For details on the media used, we refer the reader to the published research article.[23]

The utilization of industrial wastes and by-products as fermentation media for bioconversion from low-value materials to high-value materials has been explored by many researchers. Waste fibre sludge is extremely cheap and virtually inexhaustible. It can be used in BC production by employing fungal hydrolytic enzyme treatment to convert cellulose and lignin materials to sugars.[24] Research has shown that there is no significant difference in the tensile strength of BC originating from fibre waste medium and that from glucose-based medium. However, a lower crystallinity was measured for the fibre waste medium BC, compared to the glucose-based medium BC.[24] This could be due to impurities of the medium, possibly consisting of lignin degradation products or other phenolic compounds from the plant fibre.[4] Another industrial lignocellulosic waste that was explored as an alternative BC fermentation medium was corncob acid hydrolysate. However, the hydrolysis of corncob provides a substrate for *G. xylinus* to synthesize BC up to a certain point, until the accumulation of high sucrose and arabinose amounts inhibits the production process.[25] These mechanisms result in inconsistency of BC yield from different batches. A study by Huang *et al*. demonstrates the use of extracellular polysaccharides in lipid fermentation wastewater as a medium for BC production by *G. xylinus*. The BC produced from this fermentation medium had a smoother surface area with uniform porosity, but low percentage of purity.[26]

Numerous researches have been done to evaluate the potential of using industrial waste streams from beer, confectionery and biodiesel

industries for BC production.[13,22,27] The success of such an endeavour would bring dual benefits: on the one hand, wastewater treatment, and on the other hand, increased income by the generation of a new valuable product. A BC yield of 1.177 g/L was obtained by H. Zhao *et al.*,[28] close to the highest yield (1.757 g/L) obtained in other research,[23] using pullulan fermentation wastewater as low-cost substrate with high COD.

A BC with low crystallinity index and Young's modulus values was obtained in comparison with that reported in another study,[23] and the authors attributed this to the nutrient composition of the polysaccharide fermentation wastewater. Interestingly, the COD value of the wastewater was found to decrease significantly, thus suggesting an economical approach to minimizing pollution of industrial fermentation wastewater.

Recently, a maximum BC yield of 8.11 g/L has been reported when an unsupplemented distillery effluent was used to culture a novel bacterial species *Gluconacetobacter oboediens*.[29] In another study, *Gluconacetobacter sucrofermentans* B-11267 grown on an acidic by-product (thin stillage and cheese whey) from the alcohol and dairy industries was reported to give a high yield of BC, compared to the conventional Hestrin and Schramm medium. The medium with the lowest pH (thin stillage, pH 3.95) yielded the highest biomass of 6.19 g/L of BC. It was also observed that the pH of the medium increased from the initial 3.95 to 6.45, suggesting the dual benefit of the method for BC production and, at the same time, effluent treatment.[30] In some instances, improving the culture method can also increase the yield of BC production. The highest BC yield (15.6 g/L) was reported using a semi-continuous culture operation with varying parameters as 60%, 30% and 0.85 cm^{-1} for volume changing ratio, initial glucose concentration and surface area/volume ratio, respectively.[31] Further research should focus on the production of BC by utilizing low-cost substrates, such as industrial fermentation wastewater, and/or on the optimization of the fermentation conditions, medium components and additives of the established approaches (Table 1), alongside maintaining a high yield.[4,13,24,26]

Advances in microbial genomics have led to the emergence of Genome-Scale Metabolic Models (GEMs), a platform that utilizes the whole genome sequence of a particular organism to understand and predict its physiology and metabolism. It employs the use of *in-silico* approach to access and analyse the genomic data available in different databases and develop a model (using high-throughput technology and computational algorithms) that mimics the physiology and metabolism of the organism in question, so as to be able to identify the different gene annotations and their functions.[32-37] A number of successful attempts have been recorded in identifying the gene targets for antibiotic drug designing using the GEMs.[38-40] A similar approach can as well be used to understand the physiologic and metabolic complexity of cellulose producing bacteria and accordingly put forward a research effort aiming at high yield production of BC. A core metabolic model of *Kamagataebacter hansenii* has been developed based on the GEMs, using the available genome sequence of the organism. *In-silico* simulation of the model demonstrated success in predicting the minimal medium and growing abilities of the organism on different substrates, suggesting the applicability of the model in facilitating system-level metabolic analysis.[41] This could lessen the cost incurred in the trial and error approach for improving BC production.

Overly ripened and rejected fruits are often discarded as waste, since they cannot be used for human consumption. Fruit wastes normally have high content of sugars, including glucose, fructose and sucrose. Several fruit juices, such as those from pineapple, oranges, coconut, apples and grapes, have been used as culture medium for *A. xylinum* and *G. medellinensis* to produce BC.[13,20,42] With the addition of a nitrogen source, such as ammonium salt, the production of BC was elevated, thus it was suggested that fruit juices could be suitable as cheap culture medium. Nonetheless, some fruit juices have inhibitory substances that may affect BC production.[42] Moreover, fruits, such as oranges and pineapples, have a low pH, while coconut has a higher pH.[20] Thus, even though these fruits do have high sugar content, the difficulty in regulating a suitable pH of the culture medium is the biggest challenge in exploiting this source.

ENHANCEMENT OF BC PROPERTIES AND ITS WIDELY RANGING APPLICATIONS

Composites

Enhancing BC properties provides a promising path to a new generation of materials, including composites. BC composites exhibit enhanced

mechanical properties, high water holding capacity and better biocompatibility, which makes them very attractive for various fields of applications (Table 2). BC composites can be achieved through some modification processes, including treatment with a chemical reagent, the addition of nano-fillers and hybridization with other materials. The modification of BC for biomedical application mainly utilizes two major methods: the *in-situ* and *ex-situ* modification.

The *in-situ* modification employs the incorporation of additives/modifiers into the BC culture medium at the beginning of BC production, resulting in a composite material with desired properties. Meanwhile, *ex-situ* modification is done outside the growth medium (after BC purification) by either physical or chemical processes.[16,45] A sketch diagram of the modification is demonstrated in Figure 4. Physical modification can be employed to control the BC fibre porosity according to individual scaffold requirements. Chemical modification is performed to introduce some functional groups onto the BC surface to fine-tune its properties for a desired application.[45-48] In an attempt to enhance the rehydration properties of BC, citric acid was used as cross-linker to introduce carboxylic bridges within the BC fibril chains, which significantly improved not only the rehydration ability, but also the fibre porosity, suggesting its potential for biomedical application.[49]

Bacterial cellulose/poly(vinyl alcohol) BC/PVA composite was prepared by the freeze-drying method, resulting in a composite with promising properties for biomedical application, such as improved swelling behaviour and pore size with no obvious change in crystallinity.[50]

Biomedical applications

Major features of BC, such as its biocompatibility, non-toxicity and ability to promote the healing process, are of classical importance in developing BC for biomedical applications. The fabrication of BC biomedical materials will represent a significant step forward in the biomedical field, offering higher curability chances and the possibility for better treatment in various diseases and ailments. This includes treatments for cardiovascular disease through BC-based artificial vessels, the replacement of bones and cartilages, replenishing wounded cells, and more recently, drug delivery systems. Despite the promising potential that has been forecasted for BC in biomedical engineering, the exploration into its application as biomaterial is still in a preliminary state, as clearly highlighted in some studies.[45,58]

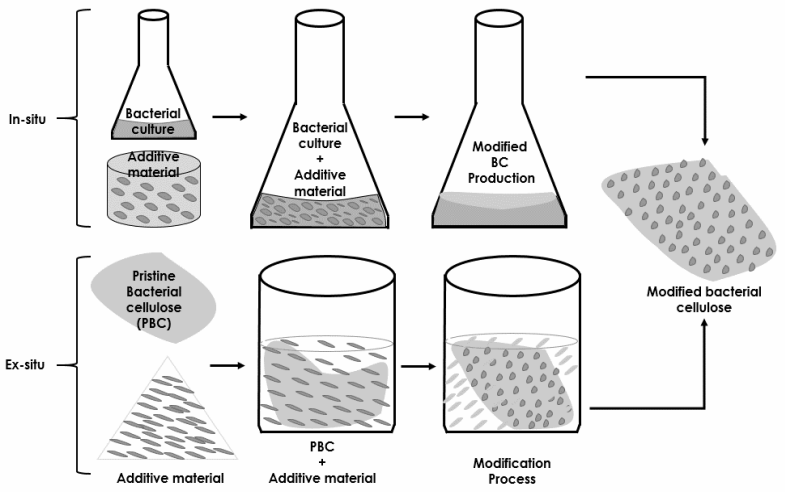

Figure 4: Sketch diagram of BC *in-situ* and *ex-situ* modification methods

Table 1
Various carbon sources used as culture media for low-cost production

Species	Medium for carbon source	BC production (g/L)	Advantage	Disadvantage	References
Acetobacter xylinum NBRC 13693	Fruit juices	0.65	- Fruit wastes contain high sugar content	- Some fruit juices contain inhibitory substances for BC production	[30]
A. xylinum TISTR 893	Coconut juice	453.33 576.66	- Consistent BC quality among different strains; - High yield	- Difficult to regulate the pH of the culture medium	[18]
A. xylinum TISTR 998	Pineapple juice	553.33 546.66			
A. xylinum TISTR 975		243.33 520.00			
Gluconacetobacter xylinus + *Trichoderma reesei* (fungi)	Waste fibre sludge	10-11	- Cheap substrate	- Low crystallinity; - Requires 2 step fermentation (first with fungi in SSF)	[22]
Gluconacetobacter xylinus	Wastewater after lipid fermentation	0.659	- Simultaneously degrades COD; - Low cost	- Slow BC yield and COD* reduction after fermentation	[24]
	Acetone-butanol-ethanol (ABE) fermentation wastewater	1·34	- Small influence on BC structure	- Low COD* degradation	[31]
Gluconacetobacter xylinus	Corncob acid hydrolysate	4	- Relatively high crystallinity; - Great water holding capacity	- BC yield was unstable for different batches	[23]
Gluconacetobacter medellinensis	Sugar cane juice and pineapple residues		- Inexpensive carbon source		[32]
Gluconacetobacter hansenii CGMCC 3917	Waste beer yeast	7.02			[25]
Komagataeibacter sucrofermentans DSM	By-product from biodiesel and confectionery industries waste	13.3	- Produced high BC concentrations		[12]

*COD: chemical oxygen demand

Table 2
BC composites with enhanced properties and their applications

Biomedicals	Enhanced properties				References
Filler/2nd phase component	Water holding capacity (%)	Tensile strength (MPa)	Young's modulus (MPa)	Others	
Calcium phosphate	N/A	N/A	N/A	Improved biocompatibility and high biodegradability	[33]
Collagen I	206	84.6	880	Promotes osteoblast development	[34]
Alginate	N/A	0.54	0.04	Non-toxic and promotes fibroblast attachment and growth	[35]
Silk sericin	high	0.6	<0.03	Biocompatible and enhanced fibroblast proliferation	[36]
Acrylic acid hydrogel	6000	1.39	377.74	Enhanced neo-vascularization, re-epithelialization	[37]
Poly(3-hydroxybutyrate)	N/A	N/A	2.0	Microstructural surfaces that encourage chondrocyte growth	[38]
Sodium carboxymethyl cellulose	N/A	N/A	N/A	Acts as carrier in drug delivery systems	[39]

Bone tissue engineering

Several BC improvements have been performed to turn it into an alternative to ceramics. BC has been known to consist in a packed fibrous structure, which forms matrixes that are able to capture various types of minerals needed to form bone-like crystals. Through the biomineralization process, BC matrixes can be deposited with calcium-phosphate (Ca-P) crystals, which are almost similar to native hydroxyapatite to improve its absorption capacity.[59] The deposition of Ca-P can be enhanced through simulated body fluid (SBF) treatment.[51] Bioabsorbable BC composites have been demonstrated to act as carriers, supplying Ca-P for bone defect repair in mouse osteoblast cell culture.[60] The biodegradability of the enhanced BC-Ca-P composite was improved, which provided not only mechanical support to the bones, but also a substrate to osteoblast cells through its biodegradation.[51] Bioactive inorganic particles, such as hydroxyapatite, are used together with BC to promote osteoblast growth. Research by Saska *et al*.[60] has demonstrated the effectiveness of bacterial cellulose-hydroxyapatite (BC-HA) membranes in *in-vivo* bone regeneration in bone defects (rat tibiae). Moreover, even after 4 weeks, the BC-HA membranes did not stimulate any inflammatory reactions and proved to have good biocompatibility.[60] The incorporation of BC-HA with osteogenic growth peptide (OGP) demonstrates the progression of bone regeneration, although it only lasted for fifteen days.[61] Functionalized multi-walled carbon nanotubes (MWNTs-COOH) were used to reinforce the mechanical properties of BC and then fabricated into a (BC/MWNTs-COOH) scaffold. Human osteoblast cells were used to test the cell viability, adhesion and proliferation on the scaffold. Viable osteoblast cells attached to the scaffold, which made it clear that the test material had a favourable interaction with the cells and can potentially be used for bone regeneration.[62] Another BC composite was formed from bacterial cellulose and collagen (BC-COL), where the BC membrane was subjected to esterification of free –OH groups through chemical treatment. Then, type I collagen was cross-linked to the esterified OH-groups using 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide. BC-COL displayed better flexibility, which gives an advantage in moulding the structure during surgical procedures.[52] Cellulose micro- and nano-hydroxyapatite (cellulose/μHA and cellulose/nHA) composite scaffolds developed for bone tissue engineering scaffold also showed an improvement in cell adhesion, increased metabolic activity and osteoblastic gene expression.[63]

Vascular graft

Blood vessel replacement is crucial in treating cardiovascular disease. Surgical treatment for cardiovascular disease requires the replacement of a blocked blood vessel either with another vessel from other parts of the patient's body or with artificial vascular prostheses. However, synthetic blood vessels made from polymers, such as polytetrafluoroethylene, are only suitable for replacement of large blood vessels ranging between 6-10 mm in diameter. Interestingly, BC nanofibre is not only capable of forming a tubular structure with a diameter less than 6 mm, but also has excellent biocompatibility and hemocompatibility, and therefore can serve as an alternative to the current artificial vascular grafts.[64,65] To enhance BC features for vascular graft application, researchers have integrated certain percentages of polymers, such as polyvinyl alcohol (PVA) and poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS), into the BC matrix. The PVA-BC composite presented significant improvement in terms of its tensile strength, Young's modulus and water permeability. The assessment of the PVA-BC composite *in vivo* indicated good hemocompatibility, with low activation of platelets and Factor XII.[65] Factor XII is responsible for thrombin activation pathways, which could lead to thrombus formation (blood clotting). BC-PEDOT:PSS in mice fibroblast cells showed good biocompatibility and hemocompatibility, as demonstrated by Khan *et al*.[66] With the use of BC-PEDOT:PSS, the formation of the filopodia structure surrounding the implanted area was observed just 3 days after implantation. This is a good indicator of cell adhesion and fibroblast growth, which assist in the adaptation of artificial vessels.

Besides synthetic polymers, natural ones, such as alginate, have been studied to make alginate-BC composites for vessel construction. Alginate is widely used in medical fields for drug delivery since it is biocompatible, low-cost and convenient to be subjected to gelation.[67] Experimentation of alginate-BC on mouse fibroblast cells (L929 model) revealed no cytotoxicity. Based on the report, the alginate-BC composite promotes the

proliferation of fibroblast cells and assists in migration and adhesion of new cells after 21 days.[53]

One of the drawbacks of using biomaterials to produce artificial vessels consists in the possibility of blood clotting in the vessel. Foreign biomaterials react with body fluids and release polyanions, which trigger the activation of platelets to bind to the polymer surface and stimulate a cascade of Factor XII.[64] To reduce the risk of activation of the coagulation factor, a new strategy is currently explored by coating the BC composites with a tripeptide of Arginine-Glycine-Asparagine (RGD). The mechanism behind this modification by the RGD tripeptide coating is to surge endothelialization of the synthetic vessel and help in reducing the thrombogenicity.[64]

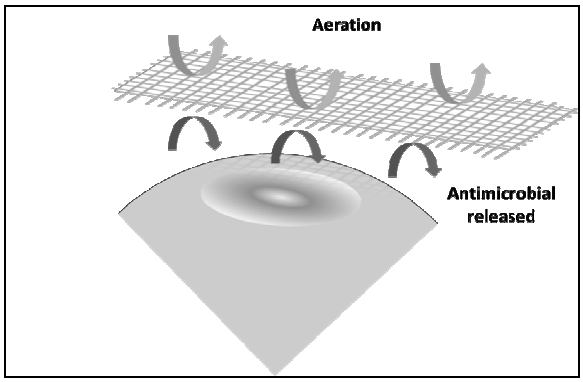

Figure 5: Illustration on BC properties assisting in wound healing

Wound dressing

BC films are suitable materials to be used for wound dressings as the ultrafine network of BC could provide an optimal healing environment (Fig. 5). The biodegradability characteristic of BC leads to reducing scarring and increasing the recovery rate of epithelial cells, thus BC reduces the time required for healing. The addition of antimicrobial agents into BC will enhance the BC properties, making it even better suited for wound dressings. The use of benzalkonium chloride as an antimicrobial agent for BC has been evaluated.[68,69] Mohite *et al*., reported the antibacterial activity of benzalkonium chloride BC film against *S. aureus* and *E. coli*, with a gradual release of up to 90% in 24 hours, while in the approach of Wei *et al*., a native BC film was soaked in benzalkonium chloride solution to allow adsorption of benzalkonium chloride cation within the ultrapores of the BC matrix. Through biodegradation of the BC-benzalkonium chloride film, the benzalkonium chloride cation is slowly released, which inhibits the growth of gram-positive (*Staphylococcus aureus*) and gram-negative (*Bacillus subtilis*) bacteria. It was recorded that 66% of the adsorbed cation was released in a period of 24 hours.[68] Cross-linkage between the BC matrix and chitosan also gives the same inhibition effect. The growth of both *S. aureus* and *B. subtilis* was significantly inhibited. Histological tests on mouse skin revealed faster regeneration of epithelial cells when the wound was treated with BC-chitosan.[70] BC microparticles were grafted with polyacrylamide using the microwave irradiation method. The composite exhibited an improvement in properties, such as water uptake, porosity and thermal stability. Testing the composite *in-vitro*, using L929 cell lines, and *in-vivo* Sprague-Dawley rats demonstrated cytocompatibility and an accelerated re-epithelialization and fibroblast proliferation, respectively.[71] BC and silver nanoparticles (AgNPs) composite was fabricated and intended to serve as antimicrobial wound dressing. The BC/AgNPs membrane displayed a broad spectrum of antibacterial activity against *E. coli* and *S. aureus*, with no toxicity reported and this was attributed to the slow release of the AgNPs from the BC membrane.[72] In another research, two forms (porous and homogeneous) of

carboxymethylcellulose (Hcel®NaT) were modified with fibrin and seeded with dermal fibroblast cells to enhance cell adhesion and proliferation. Although it was found that cell adhesion and proliferation was higher on the homogeneous form than on the porous one, the composite was envisaged to accelerate wound healing process.[73] For the first time, zinc oxide nanoparticles were incorporated into BC membrane by the matrix assisted pulse laser evaporation (MAPLE) method. It was reported that the composite of BC/ZnO showed excellent antibacterial activity against *E. coli* and *B. subtilis* and good biocompatibility with human fibroblast cells, suggesting its applicability as wound dressing material.[74]

Apart from using antimicrobial agents, enriching BC matrixes with materials that have wound healing properties, such as silk sericin, has been investigated. Silk sericin is widely known to have mitogenic and cytoprotective effects. Thus, the impregnation of a BC-matrix with silk sericin would augment wound healing and encourage fibroblast proliferation.[54] Lyophilization and thermal cross-linking methods were used to interconnect BC with glucose, which resulted in a fine 3D spongy composite with improved mechanical strength, degree of swelling and high porosity. The resulting composite was then tested *in vitro* for biocompatibility and cytotoxicity against "Vero cells" and was found to be compatible and non-toxic, thus suggesting its applicability in tissue engineering, as well as wound dressing.[75] In addition to wound healing materials, composites of BC and acrylic acid have also been reported to be able to accelerate the formation of keratin and hair follicles after two weeks of treatment.[55] The composites promote faster wound healing by enhancing the epithelialization process, thus accelerate fibroblast proliferation.[55]

Remodification of the BC/acrylic acid hydrogel to include epidermal keratinocytes and dermal fibroblast cells of human origin has also been reported to accelerate burn wound healing on mice, compared to the use of the hydrogel alone, which is related to its ability to induce a greater deposition of collagen around the treated area.[76] In a similar advancement into the use of BC as potential wound dressing material,[77] it was reported that BC membrane enrichment with lidocaine enhances the healing process in third-degree burn wounds on rats. A bacterial cellulose-copper (BC-Cu) nanocomposite, fabricated using different hydrothermal synthesis and tested *in vitro* for antimicrobial activity against *E. coli*, *S. aureus* and *Salmonella*, recommended its potential usage as a wound dressing material.[78]

Replacement of cartilage

Cartilage damage occurs commonly among elders and sportsmen, resulting in loss of function of the joint and/or muscle. As the ability of matured cartilage to regenerate is reduced with ageing, transplantation is the only option for cure. The development of BC scaffolds to fabricate cartilage offers a solution in treating damaged cartilages. However, the use of BC for scaffolding artificial cartilage is challenged by the size of its micropores. The range between 0.1 and 0.5 µm is considerably restricted for cell penetration and adhesion during cultivation of scaffold construction.[56,79] A method described by Yin *et al*. suggested using agarose microparticles during the cultivation of BC to control the size of the micropores during formation. The results revealed that, in the presence of these agarose micropores, the sizes of BC micropores were increased, thus the micropores were a thousand times larger (300-500 µm) than the control group.[79] Another novel technique was developed by Akaraonye *et al*. for BC/poly(3-hydroxybutyrate) composites, and involved the use of sucrose from grains as a porogent agent to regulate the micropore sizes through the compression moulding process.[56] The micropore sizes are fundamentally important in sustaining the growth of chondrocytes to shape a new tissue surrounding the damaged cartilage.

Drug delivery systems

Taking advantage of the biodegradability and high water holding capacity of BC, researchers have been attempting to apply it in drug delivery systems. Ibuprofen is the first model drug to be used in the exploration of BC-sodium carboxymethyl cellulose composites application in drug delivery systems.[12] In this experiment, a mathematical model was developed to study the mechanisms and kinetics of the ibuprofen-sodium released. Based on the mathematical model, the rate of ibuprofen-sodium released is inversely proportional to the BC content. This biopolymeric carrier can thus be used as an innovative drug delivery system, which might change the future of drug administration.[57] Coating with poly(lactic acid) (PLA) has been proved to enhance the antibacterial loading capacity and physical properties of BC as graphene oxide did.[80,81] The

coated BC/PLA film loaded with benzalkonium chloride (BAC) was found to inhibit the growth of *S. aureus* and *E. coli* in a slow and steady release pattern.[82] The exploration into the use of BC in drug delivery systems is deemed to be at an initial stage. However, several researchers have suggested, in their preliminary studies (*in vivo* and *in vitro*), the potential of BC as a promising biopolymer in transdermal drug delivery systems.[83-87]

CONCLUSION

Bacterial cellulose is a natural resource that can be efficiently exploited to meet various industrial needs. The high crystallinity, biocompatibility, high water absorption capacity, excellent thermal stability and the mechanical strength of BC are its remarkable features that make it stand out among other polymeric materials. Although research efforts have been made to demonstrate the value of BC for various applications in biomedical engineering, not much information has been documented, and actually, more inputs are still needed, especially with regard to drug delivery systems. Challenges associated to large-scale production of BC, with regard to its costs, have become an issue abating the awareness and interest in BC in related industries. Further research efforts are necessary to explore cheaper production techniques and/or optimize the fermentation conditions, medium components and additives of the existing approaches.

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