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REVIEW

Bacterial cellulose in the field of wound healing and regenerative medicine of skin: recent trends and future prospectives

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Abstract In this overview, we focused on the bacterial cellulose (BC) applications, described in recently published scientific papers, in the field of skin regenerative medicine and wound care industry. Bacterial cellulose was proven to be biocompatible with living tissues. Moreover, its mechanical properties and porous structure are considered to be suitable for biomedical applications. It is due to the fact that porous structure of bacterial cellulose mimics the extracellular matrix of the skin. Moreover, it can also hold the incorporated drugs and other modifiers, which can modulate its properties improving the bacterial cellulose antimicrobial activity which is rather poor for native BC. Bacterial cellulose reveals high hydrophilic properties and never dries, which is a desired property, because it was proven that wounds heal better and faster when the wound is being constantly moisturized. This characteristic of bacterial cellulose indicates that it may successfully serve as wound dressings and skin tissue scaffolds.

Keywords Bacterial cellulose · Antimicrobial activity · Synthetic polymers · Wound dressing · Skin tissue scaffold · Skin substitute

Introduction

Many materials, natural and synthetic or their composites and blends have been used as wound dressings and skin repair [1–4]. At early gestation stage the injured fetal tissues can be completely recovered, without fibrosis, in a process resembling

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regeneration [5]. Unfortunately, in human adults, this ability of wound repair process commonly leads to a scar formation [6]. Large area skin defects are the main concern in clinical treatment. Human skin transplantation can serve as a solution for the wounded tissues and is commonly used method to heal such surfaces. But there is simple restriction in form of limited amount of skin that can be transplanted. That is why the development of the new skin substitutes, as well as modern wound dressings and skin tissue scaffolds is continuously rising [7]. Suitable proposition for such applications may be cellulose obtained by bacteria in biotechnological process.

Cellulose is a well-known natural polymer, which is the most abundant, inexpensive and readily available carbohydrate polymer in the world [8]. It is also widely used in the biomedical field [9]. There are four main distinguished sources of cellulose. First and most traditional source of cellulose is its extraction from plants and their wastes. In this case, the polymer contains hemicellulose and lignin. To obtain a pure product, clean cellulose, it has to be subjected to unhealthy chemical processes applying harsh alkali and acid treatment [8].

Next two sources of cellulose are less commonly known and include the enzymatic in vitro synthesis starting from cellobiosyl fluoride, and the chemosynthesis from glucose by ring-opening polymerization of its benzylated and pivaloylated derivatives [10–12]. The last source is related with the biotechnological processes corresponding to biosynthesis of cellulose by different microorganisms, including bacteria (e.g., *Gluconacetobacter xylinus*, formerly called *Acetobacter xylinum*), algae, and fungi [9].

Bacterial cellulose [what is equal to microbial cellulose (MC)] is a polysaccharide, produced by Gram-negative, rod-shaped and strictly aerobic bacterium. It is a linear polymer made of glucose molecules linked by β(1–4) glycosidic linkages (Fig. 1). BC is commonly used in food [8] and paper industry [13–15].

In the field of regenerative medicine and wound healing BC is recently a new proposition, which is being constantly developed. There are some investigations

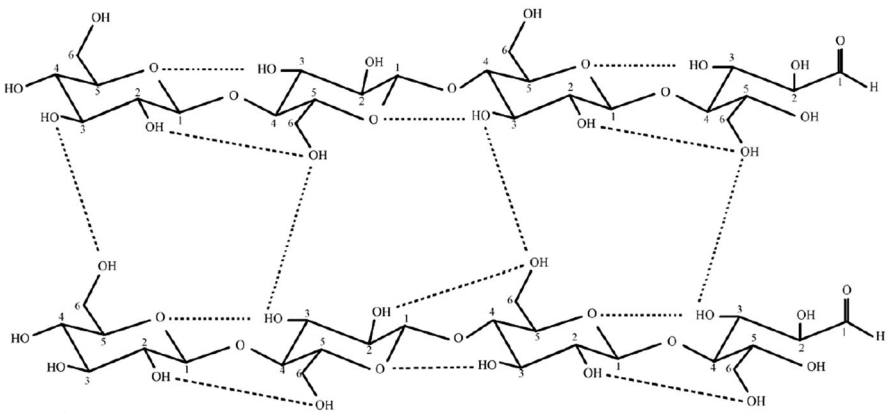

Fig. 1 Scheme of BC structure presented by Festucci-Buselli et al. [16]

revealing the application of bacterial cellulose as a tissue engineering scaffold of cartilage [17, 18], vascular grafts [19–22] nerve regeneration [19], dental implants [23–25] as well as wound dressings [26, 27] and drug delivery systems [28, 29].

Regardless of the identical chemical composition of the plant and bacterial cellulose they differ in the mechanical strength and microstructure. BC has better mechanical properties like higher tensile strength and Young's modulus (in the wet state), higher water-holding capacity, higher crystallinity [30], as well as ultrafine network structure of non-aggregated nanofibrils, availability in an initial wet state and biocompatibility [12, 19, 31, 32]. Herric et al. reported that the diameter of the nanofibrillar cellulose may be in the range of 5–50 nm, while the fiber length can exceed 1 μm [33]. But presently these values were changed, and BC diameters may be anywhere between 3 and 50 nm, while the length of the fibers can reach 10 μm [15, 34]. Nashiyama et al. noted that bacterial cellulose have high elastic modulus (∼140 GPa) [35], but the potential Young's modulus that BC fibers can achieve had value of 172.9 GPa [36]. The elongation percentage ranges between 0.8 and 2.1 %. Matsuo et al. reported high tensile strength (2–3 GPa) of BC [37], while study of O'Sullivan from 1997 reduced this range to 91–256 MPa [36] which occurred to be dependent on the preparation method. It is important to emphasize that obtained nanofiber bacterial cellulose network is highly porous, which shows selective possibility to incorporate in its structure other substances that will expand the scope of bacterial cellulose applications [38].

Large advantage of bacterial cellulose is its very high purity. In comparison to plant cellulose it does not contain lignin, hemicelluloses, pectin, and waxes. Moreover, its fabrication is not connected with harsh chemical usage, which is suitable for green chemistry requirements.

Bacterial cellulose is a natural biomaterial, produced by strains of the mentioned bacterium *Gluconacetobacter xylinus* (Fig. 2), at both static and dynamic cultures. Under these conditions different forms of cellulose can be produced. Tanskul et al. reported that under static conditions the three-dimensional interconnected reticular pellicle may be obtained and by applying agitation or stirring, which is equal to dynamic conditions, sphere-like cellulose particles (SCP) may be obtained [39]. The static process of bacterial cellulose formation is regulated with oxygen amount in culture medium and the yield depends on the carbon source concentration moderately [8, 40]. The disadvantage is synthesis of BC, which is limited to the downward pellicle growth, which entraps all bacteria [8]. Bacteria occur inactive due to insufficient amount of oxygen present in culture medium [41]. Semi-continuous process in static condition is recommended at industrial scale as it manages to increase BC productivity compared to continuous process [42]. Unfortunately, most of cellulose used in commercial purpose is still generated through agitated fermentation [8] as so in continuous dynamic process. There is also the disadvantage of bacterial cellulose culturing medium, which is not very efficient and expensive. The researchers are trying to propose novel sources for carbon supply for BC production. Their studies focus on agriculture waste and industrial by-product as potential medium [42–44]. Some of them have been proved to serve as beneficial sources of carbon for BC fabrication, such as beer waste [45], dry oil mill residue [44], grape skin [46] and maple syrup [47]. This decreases also the

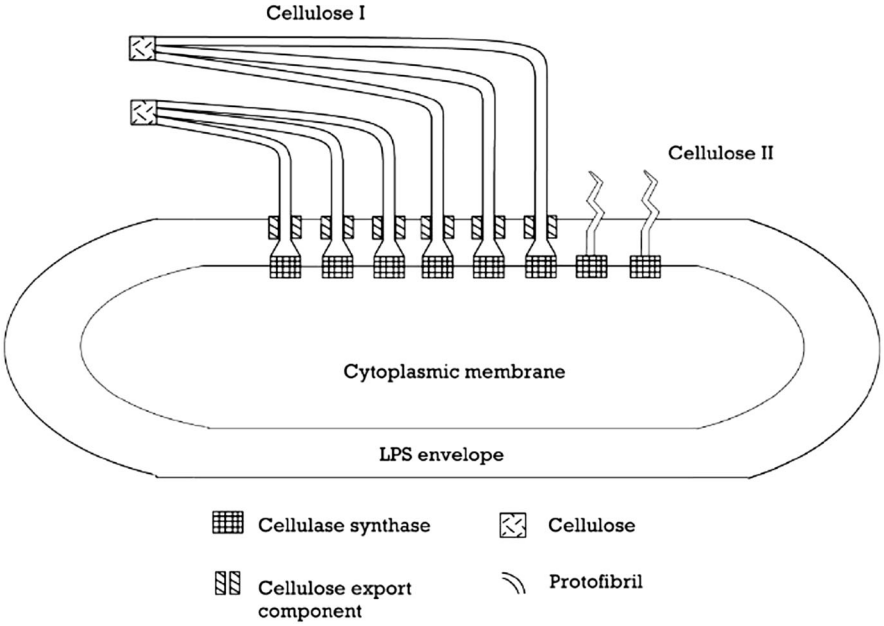

Fig. 2 Scheme of bacterial cellulose microfibrils production by *Acetobacter Xylinium* [48]

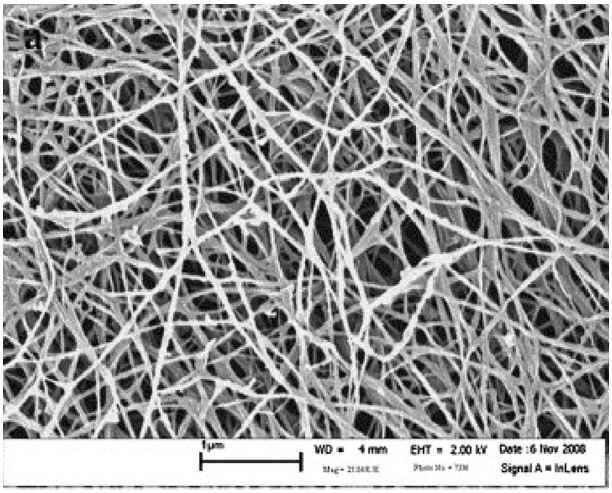

Fig. 3 The bacterial cellulose fibers obtained by Szot et al. [49]

environmental waste disposal [8]. The image of bacterial cellulose nanofibers is presented in Fig. 3, while Fig. 4 shows its modification to be used as a cellulose gel.

In this overview, we focused on the bacterial cellulose applications (BC) related with skin regenerative medicine and wound healing, which were being proposed lately in the literature data.

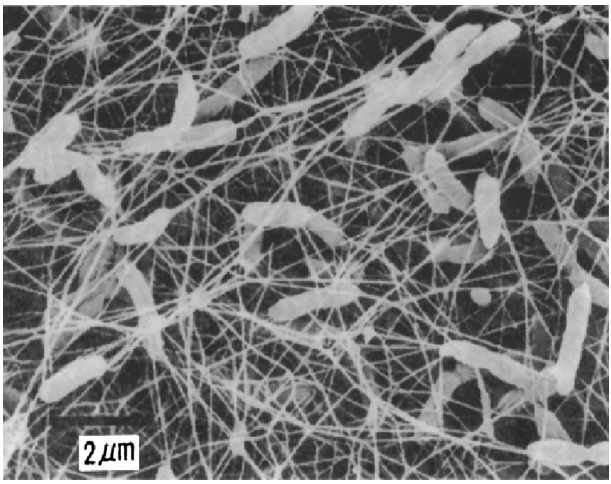

Fig. 4 The SEM image of freeze-dried surface of bacterial cellulose gel presented by Iguchi et al. [13]

Bacterial cellulose biocompatibility

Biomaterials used in the field of regenerative medicine have to be biocompatible. It means that they cannot cause permanent inflammation of the tissues [50]. BC was characterized as a biocompatible material and it was studied with this respect by some authors; for example, Backdahl et al. team that performed the BC in vivo studies. They implanted BC subcutaneously into rats and found no macroscopic signs of inflammation around the implants. There were no fibrotic capsules or giant cells. Fibroblasts infiltrated the bacterial cellulose, which was well integrated into the host tissue and did not elicit any chronic inflammatory reaction [51] which was confirmed by Helenius et al. study [52]. Bacterial cellulose was also proven to be the optimal material for skin tissue repair since it showed that BC can provide a constant moist environment to a wound (BC nanofibers can hold up to 200 times their dry weight in water without lignin and hemicelluloses present in its structure [38], which is beneficial for wound healing [53]). Moreover, the BC applied as a wound dressing shows good cytocompatibility and histocompatibility [54]. Fu et al. biosynthesized the uniform BC films, which were divided as well-cultured and normal BC films. It was indicated that they display the slight differences in surface area and porosity. The surface properties of uniform well-cultured BC were assigned to the influence of the bacterial growth microenvironment. It occurred that the behavior of bacteria in well plates seems to be important for the alignment of the BC nanofibers. The cell evaluation studies confirmed the biocompatibility of BC films. They had no toxic effects on the survival of mouse embryonic fibroblasts (3T3 line) cultured on their surfaces and the surface of BC was beneficial for cells' attachment and proliferation. The in vivo studies performed at large area of skin revealed better tissue regeneration and faster healing in the BC group [5]. These studies of BC biocompatibility are consistent with those performed by Backdahl et al. and Helenius et al.

Bacterial cellulose for wound moisture

Bacterial cellulose has been proven to be a biocompatible biomaterial, which promotes cells' attachment and proliferation in vitro, as well as improves tissue regeneration in vivo. Unfortunately this is not enough if BC has to serve as a wound dressing or skin tissue scaffold. Wound dressing should maintain the temperature of the wound bed (not evaluated) and has to accelerate the process of wound healing, as well as reduce the debris influence on the healing process. The suitably designed dressing would also preserve the gaseous exchange and would protect the newly restored tissue [26, 55]. Nowadays designed dressings should also reduce patients' pain (by covering and protecting ends of the nerves) and discomfort associated with wearing the dressing [56].

Moreover, wound dressing should maintain the suitable wound hydration. It was well documented that wound with constant moisturized environment heals faster [38]. Despite bacterial cellulose's hydrogel property, once it gets dry it exhibits poor rehydration ability. Due to this fact, Chen et al. tried to improve this BC ability. To enhance the rehydration of obtained bacterial cellulose, Chen et al. proposed its modification with hydrolyzed gelatin peptides (HGP) and/or hydroxypropylmethyl cellulose (HBC). The HGP, of molecular weights below 9 kDa, were obtained by hydrolyzing gelatin with a combination of 1 % alcalase and 1.5 % pronase E. There were two methods used for incorporation of modifiers. First method was immersing the BC template in the proper solution and the second method was adsorption. Such obtained nanocomposites (HGP/HBC) exhibited an improved rehydration ratio than composites prepared with native gelatin. The SEM analysis revealed that native gelatin and HGP penetrated the bacterial cellulose network in composite films independently from used incorporation method of modifiers. It should be emphasized that gelatine derivative caused high hydrophilicity of composites (180 % at a HGP/HBC ratio of 4.5:1 (w/w) when prepared with the use of adsorption method) [57].

Bacterial cellulose with improved antimicrobial property

The main problem, which occurs during the tissue healing, is wound infection. Monitoring of such wound state is very important not only to prevent further secondary infection but also to maintain a suitable healing process [58]. The infection caused by bacteria is visible as exudation around the wound site [59]. The modern wound dressings are designed to prevent an inherent antimicrobial effect by eluting germicidal compounds. They have been developed as a response to the commonly known problems, which occur in case of healing wounds with the use of ointments and creams [60]. There were many solutions proposed as hydrogels, hydrocolloids, films, gauzes, alginates, biologics and foams to overcome these problems [61].

The major drawback of applying BC as a wound dressing is its poor antimicrobial activity [26], which means that it cannot guarantee that the wound infection would

not appear. It is especially important in case of surgical procedures, traumatic injuries of the skin and treatment of burn wounds [62]. In the recent literature, one can find the results of studies on BC modification to improve its antimicrobial property. There are literature data about incorporation of copper [63] or silver [26, 64–66] to form copper/silver–bacterial cellulose nanocomposites (synthesis carried out in situ), forming the silver chloride–BC membranes [67], soaking the dry cellulose in benzalkonium chloride [68], blending the BC with chitosan [69], which have proven antimicrobial activity against microorganisms [70–72], or with gelatin [69]. The different direction is forming bacterial cellulose biocomposites by fabricating the bacterial cellulose–synthetic polymer composites, e.g., poly(ethylene glycol) [73].

Natural compounds as a solution for poor BC antimicrobial activity

The chitosan application was suggested by Lin et al. who obtained BC membranes and form BC–chitosan composites to achieve improved BC antibacterial property (Fig. 5). The BC–chitosan membranes were prepared by treating BC membranes with chitosan solution of 6 % chitosan concentration. SEM analysis revealed that the incorporation of chitosan into BC led to a more compact network with smaller pore size ($\sim$10 μm). The incorporation of chitosan in BC led to a more compact network with smaller pore size ($\sim$10 μm). Results from the water swelling, moisture content, water retention, and permeability tests showed that BC and BC–chitosan membranes had balanced functionality of water absorption and dehydration that helped maintain suitable moisture content for wound healing applications. These composite membranes had enhanced mechanical properties and cytocompatibility in comparison with native BC. Especially, the antibacterial evaluation revealed that the addition of chitosan into BC significantly increased the growth inhibition against *Escherichia coli* (*E. coli*)—99.9 % and *Staphylococcus aureus* (*S. aureus*)—99.9 %. The in vivo studies revealed that wounds covered with BC–

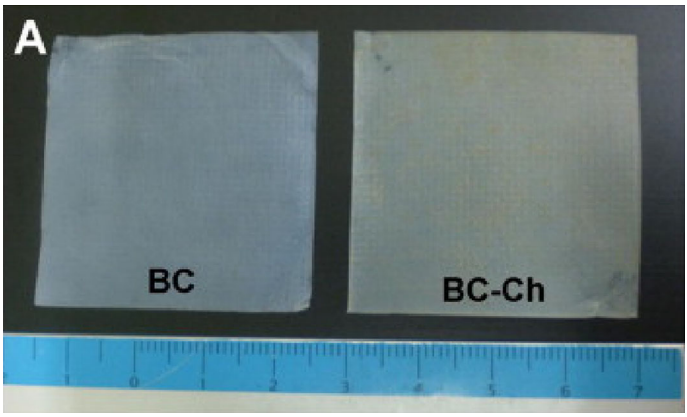

Fig. 5 The bacterial cellulose membrane (BC) and bacterial cellulose–chitosan composite membrane (BC–Ch) obtained by Lin et al. [67]

chitosan composite had shorten healing time than those treated only with BC or as those covered with commercial Tegaderm hydrocolloid or transparent films. These results demonstrated the good potential of such composites in wound treatment [74].

On the other hand, the latest study of Lin et al. proposed sodium alginate sol (SA) in combination with BC to improve BC properties. In this case they reported a new method for obtaining semi-interpenetrating polymer network of SA–BC composite. To achieve semi-IPN, the BC specimens were crushed and went through carboxymethylation. As a result there was obtained the carboxymethylated-bacterial cellulose (CM-BC) compound, which was crosslinked in the sodium alginate sol with the use of Ca^{2+} ions, which was followed by freeze drying process. The mechanical properties of such composite were once again significantly improved in comparison with native BC. Pore size of composites was in the range of 80–600 μm. The swelling capacity was enhanced with the increase of bacterial cellulose content up to 212 %, as well as thermal properties, which revealed the increment of the onset degradation temperatures from 146 °C for native sodium alginate gel up to 187 °C for its composites with bacterial cellulose. Moreover, the possibility to introduce drugs into composite structure was identified which would allow to use this composition as a wound dressing material [55] of improved BC antibacterial activity in case of suitable substances application.

Drugs as a solution for poor BC antimicrobial activity

Bacterial cellulose, as it was mentioned earlier, has fibril network, which possesses structure considered as similar to the extracellular matrix (ECM) of the human skin. That is one of the reasons why BC is being considered as a suitable material for wound dressing as well as may serve as a skin tissue scaffolds for burned wounds. The BC membranes, which never dry, are highly nanoporous materials. That allows for drugs' incorporation into its structure, which will serve as a drug delivery system of controlled release and in the same time would serve as a physical barrier for environmental pathogens that may cause wound infection [38, 75]. For example, such antimicrobial substance is benzalkonium chloride, which was incorporated in the BC by Wei et al. Such dry BC film possessed good portability and good mechanical properties, as well as high water absorption capability. Moreover, such construct revealed the strong antibacterial properties especially against Gram-positive bacteria *S. aureus* and *Bacillum subtilis* (*B. subtilis*). Moreover, the sustained release profile of the antimicrobial agent was observed within at least 24 h. The preparative procedure of the antimicrobial BC dry film was simple and versatile, which causes that many other antimicrobial agents like antibiotics, silver antibiotic agents and antimicrobial surfactants besides benzalkonium chloride could also be applied according to the introduced method [68]. On the other hand, Luan et al. with the use of ultrasonication-assisted process impregnated 1 and 2 wt% of silver sulfadiazine (SSD) into BC. This substance is applied for partial- and/or full-thickness burns [76, 77]. Among many other antimicrobial agents, the SSD reveal the wide spectrum of antimicrobial activity against *Pseudomonas aeruginosa* (*P. aeruginosa*), *E. coli* and *S. aureus* [78, 79]. SSD readily ionizes to release silver ions, which intercalate into microbial DNA and form a relatively strong bonding.

Sulfadiazine interferes with many cellular metabolic processes, including DNA synthesis, folic acid pathways and the respiratory electron transport system and can also interact with thiol groups on microbial proteins [80, 81]. The most common way of SSD application is as cream and in that way it is not free from the side effects. The enhanced inflammation is most probably caused by the water-soluble cream base itself [76]. Moreover, the SSD cream used alone cannot absorb the exudates. Luan et al.'s study revealed that incorporation of SSD into BC led to obtain the suitable construct that may serve as a wound dressing and/or skin tissue scaffold. Their study indicated also that both sonication time of SSD particles, the pH value and zeta potential (the electrokinetic potential in colloidal dispersions) of SSD have great influence on the basic impregnation process and the SSD lading. In Luan et al.'s study, the optimized parameters used for sonication of SSD were found to have pH = 8.0 and zeta potential around −37 mV. The SSD–BC composite membranes indicated antimicrobial activity against microorganisms such as *P. aeruginosa*, *E. coli* and *S. aureus*. Such composition had good biocompatibility in contact with epidermal cells, and that is why it could be a promising material for wound care of burns [75].

Chemical elements as a solution for poor BC antimicrobial activity

Silver in form of nanoparticles still occupies important position of antimicrobial agents. Its germicide activity was determined towards many different bacteria, fungi and viruses [81–83]. Silver action against bacteria is multi-directional. For example: silver ions interact with the thiol groups of enzyme and proteins that are important for bacterial respiration and nutrients transport across the cell membrane as well as within the cell [84]. Silver ions may also be bound to the bacterial cell wall and outer bacterial cell, altering the function of the bacterial cell membrane [85]. Such silver ions activity may effectively prevent wound infection [86]. As each substance and compound, silver also has drawbacks. The main disadvantage of using silver nanoparticles is their aggregation tendency, which can reduce usage utility. Few solutions were proposed for that phenomenon, which are controlled deposition of metal particles through hybridization using nanoporous materials serving as templates, which preserve the well-defined spatial distribution [87]. The shape of bacterial cellulose may ease the incorporation of nanoparticles of metals [88]. Few studies were done to determine such possibility like impregnation the bacterial cellulose with silver nanoparticles by triethanolamine (TEA) [89]; NaBH4 and UV radiation [64]; moreover by application of hydrazine, hydroxyl amine or ascorbic acid [90] as well as by self-polymerized polydopamine [91] and TEMPO-mediated oxidized BC followed by thermal reduction [88]. However, anchor metallic ions on cellulose fibers generated through these methods are weak [90]. There are also some more drawbacks of silver nanoparticles' applications. It was proven that they may cause argyrosis and argyria [92, 93]. Furthermore, high concentrations of silver are toxic for mammalian cells [94]. Due to all these side effects of silver, plenty of authors still explore its application in biomedical field of wound dressings proposing the novel solutions. For example, Wu et al. developed a new method of producing silver/nanoparticle/bacterial cellulose hybrid gel membranes of improved

antimicrobial activity. They used Tollens' reagent with bacterial cellulose, what caused the stable and strong interactions between silver nanoparticles and BC template. The silver nanoparticles content was about 2.62 wt%. Obtained gel membranes revealed significant antibacterial activity against *S. aureus*. Moreover, the gel membranes indicated suitable for rat fibroblast cells attachment and growth, showing low cytotoxicity. Excellent healing effects of a second-degree rat wound model were also observed. It is important to emphasize that stimulated with silver nanoparticles wound healing revealed formation of fresh epidermal and dermis of thickness 111–855 μm, while native BC healed wounds indicated values only about 74–619 μm, and for untreated group served as a control 57–473 μm. This short-term in vivo studies (28 days) show great potential of such metal ions' combination with BC to wound healing [95]. As a continuation Wu et al. have characterized the release profile of silver ions from BC-incorporated with silver ions hybrid. This study revealed that the obtained hybrid have great sustained and controllable release profile. Silver ions release was sustained in PBS solution with only 16.5 % of Ag^+ released in 72 h, which is in large contrast to the two control groups with 78.35 and 95.39 % burst release. Regardless to the slow Ag^+ release the hybrid revealed a significant antibacterial activity with more than 99 % reduction of *E. coli*, *S. aureus*, *P. aeruginosa* which is similar to the commercial silver-containing dressings (Coloplast®) [96]. On the other hand, Maneerung et al. to achieve antimicrobial activity, impregnated silver nanoparticles into bacterial cellulose through chemical reduction by immersing it into the solution of silver nitrate ($AgNO_3 = 0.001$ M). Sodium borohydride was then used to reduce the absorbed silver ion inside of bacterial cellulose to metallic silver nanoparticles. The obtained porous structure of freeze-dried BC had three-dimensional non-woven structures of nanofibrils (50–100 nm), which were highly uniaxially oriented at the surface of BC membrane. The applied technique, of silver ions incorporation, revealed to be very simple. In case of Maneerung et al.'s study, the BC–silver nanoparticle-impregnated membrane indicated the strong antimicrobial activity against *S. aureus* and *E. coli*, which are concerned as the main bacterial cause of wound infections. Moreover, they achieved the slow continuous release of silver nanoparticles, which were further changed into silver ions in obtained physiological system, and in that form it can slowly interact with bacterial cells. That release profile may be prolonged and reduce the same time of wound healing [26]. The significant antibacterial activity has been demonstrated for nanocomposite samples, against both Gram-negative (*K. pneumoniae*) and Gram-positive (*S. aureus*) and spore-forming (*B. subtilis*) tested bacteria, by the action of silver nanocomposite samples, which were proposed by Pinto et al. Pinto et al. in their study demonstrated that controlling of Ag concentration in the nanocomposites led to bacteriostatic (inhibition of bacterial growth) or bactericidal (killing of inoculated bacteria) activities, which can be controlled, by using different synthesis methods and/or different cellulosic substrate (vegetable or bacterial origin). In this way, it is possible to control the silver releasing ratio [64].

From the obtained results it can also be concluded that by controlling silver concentration in the nanocomposites, bacteriostatic (inhibition of bacterial growth) or bactericidal (killing of inoculated bacteria) activities can be reached and modified

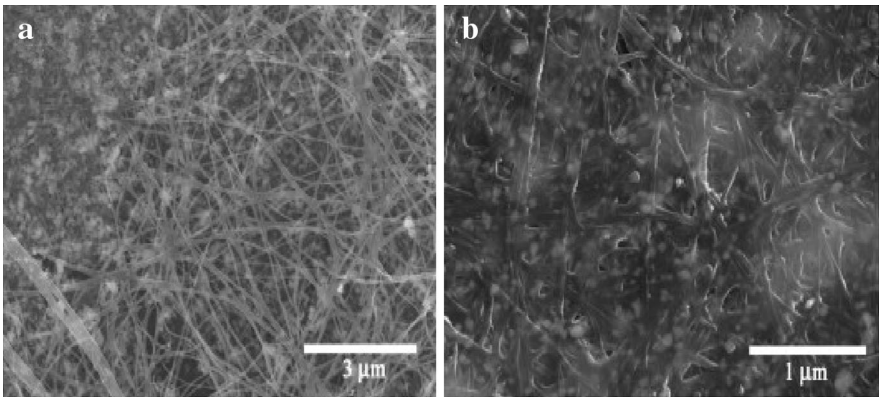

Fig. 6 The SEM images of bacterial cellulose–Ag nanocomposite presented at different magnifications: **a** 10 k×; **b** 30 k×. Both materials were obtained by Pinto et al. [57]

(Fig. 6). The silver concentration can be controlled using different synthesis methods and/or different cellulosic substrates. In this way, it is possible to control the silver releasing rate [64]. The obtained BC and BC–Ag composite membranes are presented in Fig. 7.

Another method to produce bacterial cellulose composites was proposed by Maria et al. In their study, they obtained homogeneous size distribution of silver nanoparticles (of average diameter size 30 µm) into bacterial cellulose (about 5 wt% of silver concentration in BC matrix). This new technique enabled to obtain robust, highly porous and self-sustaining structure with large surface area, which is essential to facilitate incorporation of the silver ions in the metallization process to give a high silver loading content. Furthermore, the performed in situ direct metallization method was adopted to obtain high loading content of silver ions and strong bonding forces of silver nanoparticles on the BC surface to avoid the Ag^{+} contamination problem. It was determined that the distribution of the silver particles and their sizes in the composites depend on the combination of the type of reducing and colloid protector agents used during their synthesis [66].

The efficacy of silver nanoparticles as antimicrobial agent is well established and undeniable. Although there is strong evidence that the antimicrobial activity of silver is associated with cationic release, the mechanism is not totally understood and that is why it may cause some concerns of potential cytotoxicity and genotoxicity for human cells [97, 98]. In this context, it is of interest to develop new alternatives that could replace or at least reduce usage of silver nanoparticles applied as fillers in some composite materials for biomedical applications.

Copper can provide a solution to this problem. It occurs naturally in plant and animal tissues where it participates in a number of important roles. To certain limits, the human body has mechanisms available for protection against copper toxicity at the cellular, tissue, and organ levels [99, 100]. It has been reported that copper nanoparticles have bactericidal effects comparable to silver nanoparticles in single strains of *E. coli* and *B. subtilis* [101]. Pinto et al. reported the antibacterial activity of bionanocomposites made of copper and bacterial (BC) and vegetable cellulose

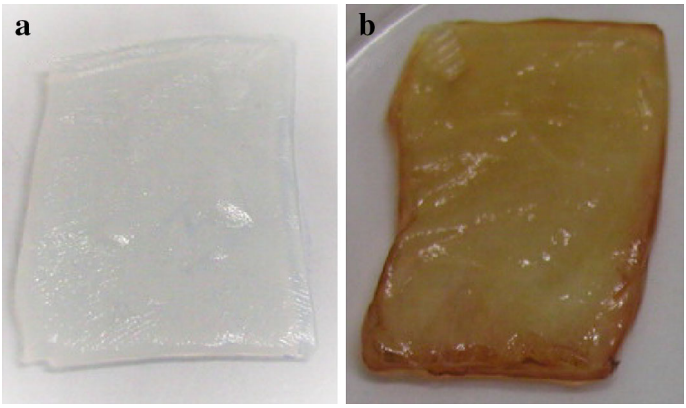

Fig. 7 The image of **a** non-treated BC membrane and **b** bacterial cellulose–Ag nanocomposite obtained by the immersion of bacterial cellulose in the silver colloids (both in wet state) obtained by Pinto et al. [57]

(VC). Pinto et al.'s recent findings showed that the chemistry of Cu nanostructures, in ambient conditions and when incorporated in cellulose matrices, depends on the morphological features of the copper particles as well as on the type of cellulose employed in the composite formulation [102]. Although bacterial and vegetable cellulose are identical from a chemical point of view, their distinct microstructures seem to influence the chemical stability of incorporated copper nanostructures, thereby with potential effects on the antibacterial properties of the corresponding composites. A series of cellulose/copper nanocomposites have been prepared by varying the type of cellulose used as the matrix (vegetable or bacterial) and also the morphology of copper nanostructures (nanoparticles or nanowires) used as fillers (Fig. 8). These composites were investigated for the first time for their antibacterial activity and it has been observed for the nanocomposite samples against both Gram-positive (*S. aureus*) and Gram-negative (*K. pneumoniae*) bacteria. Enhancement of the antibacterial activity with increasing copper content was noted. Among the morphologically distinct copper nanostructures used, the nanowires have shown less antibacterial effect than nanoparticles. Another parameter that influences the antibacterial efficiency of the nanocomposite was the structure of the cellulose fibers [63].

These results confirmed that bionanocomposites containing copper nanostructures may serve as new antimicrobial materials [63]. The summary of used chemical elements to improve BC antimicrobial property is shown in Table 1.

Bacterial cellulose dressings available in the market

Wound dressings from BC are today commonly available on the market for example: Biofill®, Bioprocess® and XCell® [53]. A commercial product Biofill®, which was a partially dried BC membrane, was developed for wound healing of

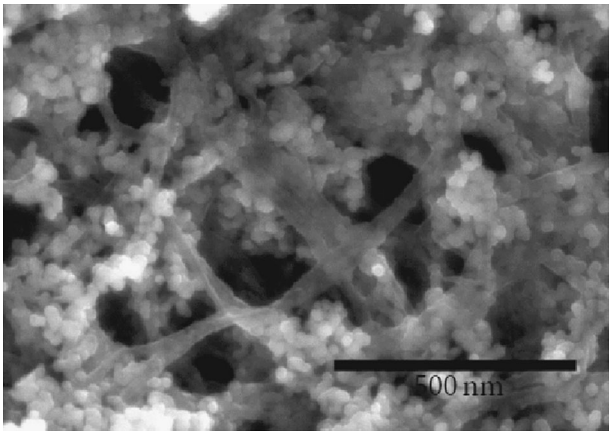

Fig. 8 The SEM image of bacterial cellulose–copper composite obtained by Pinto et al. [95]

burns and chronic ulcers. The studies revealed that Biofill® had a more effective performance than other wound dressing materials in accelerating the healing process, pain relief, etc. [27]. Another BC wound dressing called XCell® have been applied to heal chronic venous ulcers, and once again, the BC dressing showed satisfactory effect in treating these chronic skin abnormalities [103]. Park et al. performed a comparative study of other commonly know wound dressing such as Vaseline gauze and Algiste M (calcium alginate gel) to determine how does the bacterial cellulose stack up to other types of dressings, which are commonly available on the market. The BC was obtained as a strain separated from the natural fermentation liquid of domestic citrus fruit extract. Park et al. wanted to found out if cellulose from citrus is safe and innocuous to organism. To perform such study they formed bacterial cellulose films which were transplanted onto the backs of white mice to verify its biodegradability and toxicity. The BC used in this study was indicated as a biocompatible material without toxicity, which effectively biodegrades in vitro and is a safe-to-use material of high clinical application. Obtained BC accelerates contraction through the accumulation of extracellular matrix [104]. Moreover, Park et al. in their studies indicated that bacterial cellulose wound dressings may be applied to various type of wounds such as cavity, laceration, abrasion, etc. However, it cannot be disregarded that a limitation of noted differences in healing mechanism between animals and humans was observed in this study. Therefore, for further clinical applications of bacterial cellulose derived from citrus, clinical trials targeted on people have to be performed.

Bacterial cellulose composites with medical-grade polymers

Many synthetic polymers have been used in the medical field and in regenerative medicine of skin tissue and wound healing [1–4]. This group of biomaterials includes poly(lactic acid); poly(glycolic acid) and their copolymer poly(lactide-*co*-

Table 1 Chemical elements, their dimensions, content in the BC composite and antimicrobial activity noted

References	Used chemical compound	The diameter of nanoparticles	The content in the BC composite	Antimicrobial activity	
Wu et al. [89]	$AgNO_3$	10–20 nm	1.09–3.40 wt%	*E. coli* *S. aureus* *P. aeruginosa*	=99.8–100 %
Wu et al. [88]	$AgNO_3$	10–100 nm	2.62 wt%	*S. aureus*	Observed increased antimicrobial activity
Maneerung et al. [24]	$AgNO_3$	5.47 nm 6.48 nm 11.34 nm	–	*E. coli* *S. aureus*	=99.7 % =99.9 %
Pinto et al. [57]	$AgNO_3$	38 nm	5×10^{-4} wt%	*B. subtilis* *S. aureus* *K. pneumoniae*	Observed increased antimicrobial activity
Maria et al. [59]	$AgNO_3$	30 nm	5 wt%	*E. coli*	=100 %
Pinto et al. [95]	$Cu(NO_3)_2 \times 3H_2O$	36 nm (nanoparticles) 90–220 nm (nanowhiskers)	0.93–5.7 % w/w	*S. aureus* *K. pneumoniae*	Observed increased antimicrobial activity

glycolide), poly(ε-caprolactone), poly(ethylene glycol) and polyurethanes [105]. Some of the mentioned materials were used to improve bacterial cellulose mechanical properties. For example, Zhijiang et al. described the preparation of bacterial cellulose/poly(ethylene glycol) (BC/PEG) composite (Fig. 9) using the method where previously produced BC hydrogel was soaked with PEG solution (1 %), allowing the PEG molecules to penetrate the BC, followed by freeze-drying process. This method was formerly described by Alberto et al. [106]. This method occurred to be simple and effective. SEM images revealed that PEG molecules were not only coated on the BC fibrils surface but also penetrated into the BC fiber networks. Obtained composite scaffolds had interconnected porous network structure and large aspect surface. The presence of PEG affected the preferential orientation of the plane induced by the drying process of BC pellicle. That resulted in decreased crystallinity of dried BC. The thermogravimetric analysis found out that the thermal stability of composites was improved. Tensile test results indicated that the Young's Modulus and tensile strength tended to decrease while the elongation at break had slight increase. Cell adhesion study was carried out using 3T3 fibroblast cells. The cells incubated with BC/PEG composite scaffold for 48 h were capable of cell attachment and further proliferation. It indicated that composite materials revealed better biocompatibility than pure BC. Thus, suggesting that scaffolds obtained by Park et al. can be used for wound dressing or tissue engineering applications, as well as drug delivery systems [73].

In 2011, Zhijiang et al. started studies on biocompatible composites prepared by impregnation of poly(3-hydroxybutyrate) with bacterial cellulose nanofibrils. That treatment caused that micro- (30 μm) and nano-scaled (300 nm) porous structure was observed in obtained PHB/BC nanocomposite scaffolds, which is beneficial for wound healing. The SEM images showed well-dispersed BC nanofibrils in PHB matrix. Crystallinity studies indicated that BC nanofibrils may be involved in PHB molecules' crystallization and in this way modify the composite mechanical properties. The in vitro studies revealed good cell attachment. The revealed biocompatibility was more suitable for wound healing in case of BC composite than

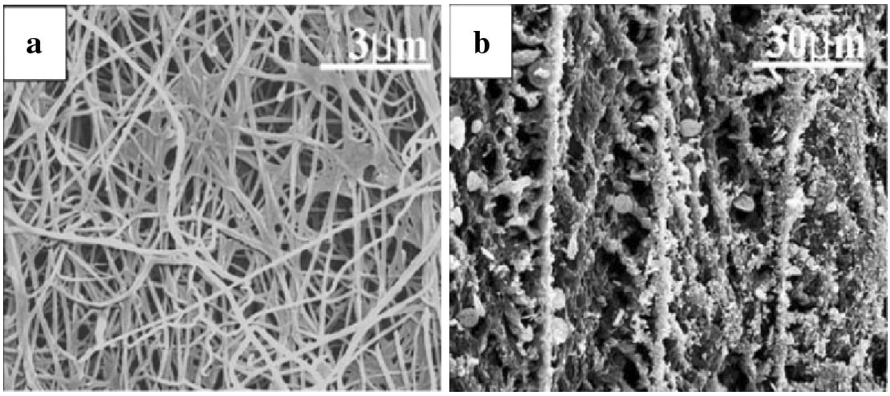

Fig. 9 The SEM images of Zhijiang et al. bacterial cellulose (**a**) and bacterial cellulose–poly(ethylene glycol) composite fibers (**b**) [66]

native PHB [107]. Zhijiang et al. continuing the studies on PHB/BC composites used poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) and obtained a novel biocomposite scaffold of suitable biocompatibility tested in vitro. The composite structure had multidistributed pore sizes (macropores of 20 μm and nanopores of 500 nm). Moreover, the increased hydrophilic property was achieved (on the contrary to native composite without bacterial cellulose), which is beneficial, as mentioned earlier, for constant wound moisturizing and its faster healing. The performed preliminary biodegradation examination revealed the improvement of its rate [108] as so such composite may be suitable for skin tissue scaffolding.

Conclusions

In this overview, we focused on the bacterial cellulose applications (BC), which are being proposed lately in the literature data related with skin regenerative medicine and wound healing. This literature research revealed that bacterial cellulose possesses a lot of advantages. It was proven to be biocompatible in studies in vitro and in vivo on rats. Moreover, it has great mechanical characteristics and porous structure mimicking the ECM of native skin. Bacterial cellulose reveals high hydrophilic properties and never dries, which is a desired property, because it was proven that wounds heal better and faster when the wound is being constantly moisturized. Few BC-based commercial products are available on the market today and serve their function as wound dressings of cavities, laceration, abrasions and also as dressings for burns and chronic venous ulcers. Due to the porous structure of BC it is possible to incorporate drugs in its structure, which can improve bacterial cellulose properties, e.g., antimicrobial activity, which is rather poor of native BC. The other possibility is to incorporate chemical elements, such as silver and copper, which show very popular trend of satisfactory results in improving antimicrobial property of BC. The other drugs and chemical elements/compounds should be studied further to improve the healing potential of such BC wound dressings and tissue scaffolds. Some studies were performed to form bacterial cellulose–synthetic polymer composites, which highly improve bacterial cellulose mechanical properties and porosity, which is suitable for biomedical applications. Other synthetic polymers should also be tested to improve the mechanical properties of such wound dressings and tissue scaffolds. The biotechnological process of obtaining bacterial cellulose should also be taken into consideration in further studies for it to be more efficient. It can happen due to new sources of carbon for BC biosynthesis. This characteristic of bacterial cellulose-based materials indicates that it may successfully serve as a wound dressing and skin tissue scaffold.

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