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Present status and applications of bacterial cellulose-based materials for skin tissue repair

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ABSTRACT

Bacterial cellulose (BC, also known as microbial cellulose, MC) is a promising natural polymer which is biosynthesized by certain bacteria. This review focused on BC-based materials which can be utilized for skin tissue repair. Firstly, it is illustrated that BC has unique structural and mechanical properties as compared with higher plant cellulose, and is thus expected to become a commodity material. Secondly, we summarized the basic properties and different types of BC, including self-assembled, oriented BC, and multiform BC. Thirdly, composites prepared by using BC in conjunction with other polymers are explored, and the research on BC for application in skin tissue engineering is addressed. Finally, experimental results and clinical treatments assessing the performance of wound healing materials based on BC were examined. With its superior mechanical properties, as well as its excellent biocompatibility, BC was shown to have great potential for biomedical application and very high clinical value for skin tissue repair.

1. Introduction

Cellulose is well known as one of the most abundant biodegradable materials in nature and has thus been the topic of extensive investigations in macromolecular chemistry. Over the past 30 years, developments in molecular biology and the application of cell systems *in vitro* have resulted in extensive exploration of the mechanisms underlying the biosynthesis of cellulose in nature. Cellulose based polymers have wide applications in tissue engineering, controllable delivery system, blood purification, sensor, agriculture, as well as water purification (Chang & Zhang, 2011). Bacterial cellulose (BC, also known as microbial cellulose, MC) is a promising natural cellulose synthesized by certain bacteria. The wide applications of BC are foreseeable.

Because of its unique structural and mechanical properties as compared with higher plant cellulose, BC is expected to become a commodity material in various fields. BC fibers have a high aspect ratio with a diameter of 20–100 nm. As a result, BC has a very high surface area per unit mass. This quality, combined with its highly hydrophilic nature, results in a very high liquid loading capacity. Moreover, its biocompatibility, hydrophilicity, biocompatibility, transparency and non-toxicity make it an attractive candidate for a wide range of applications in various fields, especially those related to biomedical and biotechnology applications (Dahman, 2009). The fibrous structure of BC consists of a three-dimensional non-woven network of nanofibrils, sharing the same chemical structure as plant cellulose, which is held together by inter- and intra-fibrilar hydrogen bonding, resulting in a hydrogel state with high strength. The biosynthetic pathways of BC, including those involving enzymes and precursors, have previously been described in detail by Chawla, Bajaj, Survase, and Singhal (2009). Such biomedical devices are advantageous in terms of their high paper-like reflectivity, flexibility, contrast, and biodegradability (Klemm, Heublein, Fink, & Bohn, 2005). Much work has already focused on designing ideal biomedical devices from BC, such as artificial skin, artificial blood vessels, artificial cornea, heart valve prosthesis, artificial urethra, artificial bone, artificial cartilage, artificial porcine knee menisci, and deliveries of drug, hormone and protein (Halib, Amin, Ahmad, Hashim, & Jamal, 2009; Oshima, Taguchi, Ohe, & Baba, 2011; Petersen & Gatenholm, 2011; Wang, Gao, Zhang, & Wan, 2010) As an intuitionistic introduction, the prospects for the various biomedical applications of BC-based materials are shown in Fig. 1.

It is clear from previous research that the materials derived from BC can provide a promising future for biomedical application. This paper reviews the applications of BC as skin tissue repair material; specifically, we summarize the researches on BC for the application of BC in skin tissue engineering. Experimental results and clinical treatments have demonstrated the effectiveness of BC-based wound healing materials. Furthermore, all the results have indicated that BC as a skin tissue material in the biomedical field will have continuing importance in the future.

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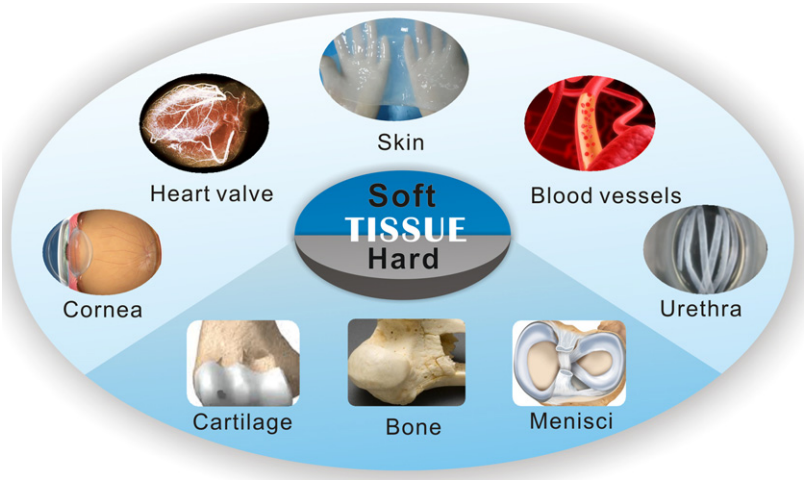

Fig. 1. Prospects for the various biomedical applications of BC-based materials.

2. Basic properties

The hydrophilic ability of BC is determined by its high water content, while only 10% out of the 99 wt% water presented in BC gels behave like free bulk water (Gelin et al., 2007). Recent studies have shown that atomic force microscopy can be used to measure the elastic modulus of suspended fibers through a nanoscale three-point bending test. Guhados, Wan, and Hutter (2005) measured Young modulus of BC fibers with diameters ranging from 35 to 90 nm at a value of 78 ± 17 GPa (Guhados et al., 2005). The value obtained (114 GPa) was higher than those previously reported, but lower than estimates from the modulus of crystalline cellulose-I (130–145 GPa) (Hsieh, Yano, Nogi, & Eichhorn, 2008).

A study by McKenna et al. showed that an increase in the fermentation time could lead to a decrease in mechanical strength, illustrated by Young's modulus first increasing and then decreasing after 96 h. BC behaves like a viscoelastic material; brittle failure has previously been reached at approximately 20% strain and 1.5 MPa stress under uniaxial tension (McKenna, Mikkelsen, Wehr, Gidley, & Menzies, 2009). Slightly enhanced tensile strength and deformation at break were obtained by increasing the molding compression pressure, while the modulus also decreased nearly linearly with increasing film porosity. This behavior was related to denser structure under increased mold compression, which reduced the interfibrillar space, thus increasing the probability of interfibrillar bonding (Retegi et al., 2010).

3. Bio-fabrication

The fabrication of a BC network sheet was attempted by heat-pressing in metal molds with a micro pattern to open a pathway to potentially versatile materials. To modify the surface of natural fibers, BC was utilized as a substrate for bacteria during fermentation of BC (Pommet et al., 2008). A structural hydrophobic similar to the "Lotus effect" was thus examined on this sheet, by introducing a micro-lattice pattern on to its surface. Indeed, the surface of the sheet was found to be more hydrophobic when the structural hydrophobic effect and the synergistic effects of heating and micro-patterning were combined (Tomita, Tsuji, & Kondo, 2009). Efficiency in the production of BC is indispensable in determining its potential applications. Carreira et al. (2011) evaluated several residues from agro-forestry industries as economic carbon and nutrient sources for the production of BC: namely grape skins aqueous extract, cheese whey, crude glycerol and sulfite pulping liquor. Agro-forestry residues were successfully used as carbon sources for the production of BC. The most relevant results were attained with wine and pulp industries residues: 0.6 and 0.3 g/L of BC (Carreira et al., 2011).

3.1. Self-assembled and oriented bacterial cellulose

Potato and corn starch were added to the culture medium and partially gelatinized in order to allow BC nanofibrils to grow in the presence of a starch phase. The BC-starch gels were hot pressed into sheets with a BC volume fraction higher than 90%. During this step, starch was forced to further penetrate the BC network. The self-assembled BC-starch nanocomposites displayed coherent morphologies (Grande et al., 2009). Five separate sets of experiments were conducted to demonstrate the assembly of nanocellulose by *Acetobacter xylinum* (*Gluconacetobacter xylinus*) in the presence of electric fields in micro- and macro-environments, which demonstrated a new concept of bottom up material synthesis through a biological assembly process (Sano, Rojas, Gatenholm, & Davalos, 2010). The maximum water holding capacity value 92.21 g/g was measured for BC formed in reactors modified with 3.0% of agar. The maximum production rate was observed after the second day of cultivation as compared to the third day of cultivation in the case of the control experiment without agar (Shah, Ha, & Park, 2010).

BC gel can be produced on an oxygen-permeable substrate such as polydimethylsiloxane (PDMS). The optimum ridge size of 4.5 μm was related to the contour length of the bacteria cells. The fracture stress (σ) of uniaxially oriented BC gel under elongation was 4.6 MPa, which was 2.3 times higher than that of the BC-air material (σ = 2 MPa) (Putra et al., 2008). The extraction and refinement of high-strength crystalline microfibril bundles (15–20 nm thick) from BC networks was investigated, as well as their morphology prior to and post electrospinning. The diameter of the fibers decreased significantly with increasing cellulose content from about 1.8 μm (1 wt%) to about 100 nm (20 wt%). The results demonstrated a significant improvement in thermal stability for the composite material. The fibers were aligned into an anisotropic nanocomposite during spinning (Olsson, Azizi Samir, et al., 2010; Olsson, Kraemer, et al., 2010).

3.2. Magnetic bacterial cellulose

Bacterial cellulose, with its porous network structure, was also used as an accelerator to precipitate Ni nanoparticles through the room temperature chemical reduction of $NiCl_2$ hexahydrate.

Interestingly, BC did not undergo any change and retained its crystal structure even after the chemical reduction reaction. The fraction of isolated superparamagnetic nanoparticles present in the composite was estimated from the saturation magnetization and found to be around 88% (Vitta, Drillon, & Derory, 2010).

3.3. Modification of bacterial cellulose

These provided new ideas for the modification of BC. A novel copolymer of polylactide and glycidyl methacrylate (PLA-co-PGMA) was prepared and used to modify the BC surface. PLA-co-PGMA was efficient in the modifications and improving the compatibility of PLA/cellulose composites. Moreover, polylactide-graft-methacryloxypropyltrimethoxysilane (PLA-g-MPS) was prepared by grafting MPS on to PLA, which was subsequently used to modify BC. The results revealed that the modified BC had a more hydrophobic nature than virgin BC (Li, Zhou, & Pei, 2010a, 2010b). Natural fibers have previously been modified for the reinforcement of polymers by producing a diblock copolymer of BC and poly(methyl methacrylate) (BC-block-PMMA) through the mechanical fracture of BC with MMA (methyl methacrylate) in vacuum at 77 K, among other methods. The radical polymerization of MMA was initiated by the mechanoradicals located on the BC surface, which was fully covered with the PMMA chains of the BC-block-PMMA (Sakaguchi et al., 2010). Polyaniline (PAni) was used to modify the BC by *in situ* nano-assembly of BC nanofibers and PAni. The electroconductivity of composite hydrogels was enhanced from 10^{-8} to 10^{-2} S cm^{-1}, which demonstrated that the BC/PAni composite was an electro-conductive hydrogel. Shi et al. (2012) reported that composites might have potential applications for flexible displays, biosensors, and platform substrates to study the effect of electrical signals on cell activity, and to direct desirable cell function for tissue engineering applications (Shi et al., 2012).

3.4. Multiform bacterial cellulose

The analysis of lyophilized sphere-like BC particles indicated that the agitation speed of the culture had an impact on the internal structure of the sphere-like particles. The smaller sphere-like particles produced at 150 rpm were hollow and their cellulose shell exhibited a layered structure. The larger particles produced at 125 rpm did not exhibit a layered structure in the central region of the cellulose, yet the outer layer was similar in structure to the particles produced at 150 rpm (Hu & Catchmark, 2010).

Phase separation phenomena in aqueous suspensions of BC nanocrystals obtained by sulfuric acid hydrolysis have also been studied. Suspensions at concentrations above 0.42 wt% separated into isotropic and chiral nematic phases with a clear phase boundary. The size of the ordered domains in the anisotropic phase decreased with NaCl concentrations in the range from 0 to 2.75 mM. At 2.75 mM, only tactoids were observed in the entire region. While at 5.0 mM, chiral nematic domains were no longer observed. The chiral nematic pitch decreased as the concentration of NaCl increased, reaching a minimum value at approximately 0.75 mM, and then increasing sharply with the NaCl concentration up to 2.0 mM (Hirai, Inui, Horii, & Tsuji, 2009). Carboxymethyl cellulose (CMC) or xyloglucan (XG) were added to the aggregated BC nanocrystals (BCNs) suspensions to minimize this problem. CMC enhanced the dispersion of BCN above a concentration ratio of 0.05. In the case of XG, enhanced colloidal stability was observed above a concentration ratio of 0.5. The results demonstrated that cellulose-based model surfaces obtained by spin-coating from CMC/BCN or XG/BCN solutions exhibited a more uniform topography and less surface roughness than the control unmodified BCN model surface (Winter et al., 2010).

4. Applications for skin tissue repair

Owing to its unique nano-scaled three-dimensional network structure, BC has high water retention, high mechanical strength, and outstanding biocompatibility, all of which enable it to serve as a natural scaffold material for the regeneration of a wide variety of tissues (Czaja, Krystynowicz, Bielecki, & Brown, 2006; Klemm et al., 2006; MacNeil, 2007; Siró & Plackett, 2010). For most repair materials, an important characteristic is their ability to absorb exudate during the dressing process, as well as during their removal from a wound surface after recovery. Traditionally, skin tissue repair materials have been absorbent, permeable materials. For example gauze, a traditional dressing material, can adhere to desiccated wound surfaces and thus induce trauma on removal. Recently, interest in cellulose produced by bacteria from surface cultures has increased steadily due to its potential for application in medicine and cosmetics (Hornung, Biener, & Schmauder, 2009). When considering the properties of BC as well as its clinical performance, the commercialization of BC for wound care seems very promising (Czaja et al., 2006).

4.1. Basic properties

Compared to plant cellulose, BC has features such as a high crystallinity, tensile strength, water absorption capacity, good permeability, biocompatibility, resistance to degradation, and a low solubility which may prove advantageous for skin tissue materials. The BC pellicle has an asymmetric structure composed of a fine network of nanofibrils similar to a collagen network. The shape of the stress–strain response curve of BC is reminiscent of the stress–strain response of the carotid artery, most probably due to the similar architecture of both types of nanofibrill networks (Backdahl et al., 2006). Culture mediums with different additives such as manitol and glycerol were used to produce BC films. The two BC films exhibited slight but not hugely significantly differences in crystalline features. In addition, it was observed that cellulose fibrils of BC in medium with manitol were thinner and reticulated to form slightly smaller porosity than those in medium with glycerol (Kaewnopparat, Sansernluk, & Faroongsarng, 2008).

The presence of a large number of hydroxyl groups provides strong advantages as the water vapor permeability of air-dried BC is subsequently quite good. By the addition of various concentrations of a single sugar alpha-linked glucuronic acid-based oligosaccharide (SSGO) to the culture media, the *in situ* modified BC showed denser fibril arrangement and decreasing pore size and pore volume with increasing SSGO concentration. The water holding capacity and water release rate increased with pore volume and pore size in *in situ* modified BC samples (Ul-Islam, Khan, & Park, 2012).

Bio-absorbability of the BC is desired to enable improved restoration of targeted tissue in wound environment. Cellulose biodegradation resulting from enzymoly-sis generally occurs in nature rather than in the human body because of the absence of cellulose degrading enzymes. In order to achieve degradation of BC, bio-absorbable BC was investigated by Hu and Catchmark (2011a, 2011b). *In vitro* studies on the use of buffers with pH values relevant to wound environments revealed that acidic cellulases from *Trichoderma viride* showed reasonable activity for pH values ranging from 4.5 to 6.0. A commercial cellulase (cellulase-5000) did not show good activity at pH 7.4, but its degrading ability increased when used in conjunction with a beta-glucosidase from *Bacillus subtilis* or a beta-glucosidase from *Trichoderma* sp. They also reported that the incorporation of buffer ingredients helped to retain the activity of the cellulases. The glucose released from degraded materials also increased from 30% without the incorporation of buffer

ingredients to 97% in the presence of incorporated buffer ingredients at the suboptimal pH environment of 7.4.

4.2. Biocompatibility

4.2.1. The biocompatibility of bacterial cellulose

BC is clearly advantageous as engineered skin tissue material. However, little information is available concerning the potential toxicity of BC-based biomaterials. The toxicity of BC nanofibers was evaluated *in vitro* through cell viability and flow cytometric assays and *in vivo* using C57/B16 mice surgeries. The microscopic morphology of the human umbilical vein endothelial cells (HUVEC) was also examined following culture in the absence of the cellulose nanofibers and with nanofibers for 24 h and 48 h. No obvious difference in morphology was observed (Jeong et al., 2010). After co-culture with fibroblasts (FB) and chondrocytes, respectively, BC compositions were implanted into nude mice. The BC co-culture composition was well integrated into the skin of nude mice. Thus, it is natural to conclude that BC was beneficial to cell attachment and proliferation under these conditions (Wang et al., 2009). The *in vitro* evaluation of the interactions between cells and BC was performed through viability staining analysis on cells grown on the biomaterial. This demonstrated that 95% of the mesenchymal stem cells aggregating to the cellulose membrane were alive, while 5% were necrotic. SEM showed that the cells were morphologically normal and attached to the cellulose membrane surface (Mendes et al., 2009). Using the improved multilayer fermentation method, BC for skin tissue repair was also produced in the research of Fu et al. (2012). Low cytotoxicity of the BC film and good proliferation of human adipose-derived stem cells on the BC film were observed. Full-thickness skin wounds were made on the backs of BALB/c mice, and subsequent histological examinations demonstrated significant fresh tissue regeneration and capillary formation in the wound area in the BC groups on day 7 compared with those commercial dressings and animal skins in other groups. These results indicate the high production efficiency of BC and thus, its high clinical potential (Fu et al., 2012).

4.2.2. The biocompatibility of bacterial cellulose-based materials

To improve the biocompatibility of BC, different extracellular matrices (ECMs: collagen, elastin, and hyaluronan) and growth factors (basic fibroblast growth factor (B-FGF) human epidermal growth factor (H-EGF) and keratinocyte growth factor (KGF)) were immobilized on to macroporous BC (Lin, Wey, & Lee, 2011; Lin, Chen, Ou, & Liu, 2011). The H-EGF and collagen-modified BC supported the growth of human skin fibroblast. The attachment of cells to biomedical materials can be improved by utilizing adhesive amino acid sequences, such as Arg-Gly-Asp (RGD), found in several extracellular matrix proteins. Andrade et al. grafted RGD on to BC films that exhibited improved biocompatibility (Andrade, Moreira, Domingues, & Gama, 2010). In order to enhance cell affinity, BC was also modified with nitrogen plasma. The treatment did not increase the wettability of the material, but increased its porosity and modified its surface chemistry, as demonstrated by the presence of nitrogen. The potential of plasma treatment for the surface modification of BC was also demonstrated (Pertile, Andrade, Alves, & Gama, 2010). Furthermore, microporous BC scaffolds have been seeded with urine-derived stem cells, which were induced to differentiate into urothelial and smooth muscle cells (Bodin et al., 2010).

4.3. Composites

To improve the positive features of BC as wound dressing material, BC can be modified through the incorporation of collagen type I. SEM images showed that the collagen molecules were not only coated on the BC fibrils surface, but could also penetrate inside BC and hydrogen bond interactions were thus formed between BC and collagen (Cai & Yang, 2011). Double-network (DN) hydrogels with high mechanical strength were synthesized from BC and gelatin. The fracture strength and elastic modulus of BC-gelatin DN gel under compressive stress were on the order of megapascals, which was several orders of magnitude higher than for gelatin gel. Similar enhancements in mechanical strength were also observed for a combination of BC with polysaccharides such as sodium alginate, gellan gum, and i-carrageenan (Nakayama et al., 2004). BC was modified with poly(3-hydroxubutyrate-co-4-hydroxubutyrate) (P(3HB-co-4HB)). The biocompatibility of the composite scaffold was preliminarily evaluated by cell adhesion studies using Chinese Hamster Lung (CHL) fibroblast cells. The results showed better biocompatibility of P(3HB-co-4HB)/BC composite scaffold than that of pure P(3HB-co-4HB) scaffold (Cai, Hou, & Yang, 2011a).

For example, the composite with 80 wt% BC/20 wt% alginate displayed a homogeneous structure and exhibited enhanced water adsorption capacity and water vapor transmission rate. Supercritical carbon dioxide drying was used for the formation of a nanoporous structure. However, the tensile strength and elongation at break of a film with a thickness of 0.09 mm decreased to 3.38 MPa and 31.60%, respectively. The average pore size of the blend membrane was 10.6 Å with a 19.5 m^2/g specific surface area (Phisalaphong, Suwanmajo, & Tammarate, 2008). The *in vitro* studies with human keratinocytes and gingival fibroblasts demonstrated that the pure BC and the BC/alginate sponges supported proliferations of the cells. However, in the wet state, only the BC/alginate sponge with 30% alginate had good tear resistance for sewing (Chiaoprakobkij, Sanchavanakit, Subbalekha, Pavasant, & Phisalaphong, 2011). Due to its many advantages in terms of skin tissue compatibility, excellent water uptake ability, high mechanical strength, and stability in both water and PBS buffer, the BC/alginate composite sponge is a promising material for use as a non-adherent hydrogel dressing.

Beside the composite with alginate, BC and gelatin were also selected to prepare membranes, and the morphology of NIH/3T3 cells grown on the surface of these membranes was examined to select the best material for the development of a biodegradable skin tissue regeneration template (Fig. 2). Membrane derived cow bone gelatin and fish skin gelatin were stronger and more flexible than those prepared from pork skin gelatin in their wet forms (Nwe, Furuike, & Tamura, 2010). To develop functional property, a freeze-dried BC film was immersed in a benzalkonium chloride solution: a cationic surfactant and antimicrobial agent, followed by another freeze-drying step. It was shown that the drug-loading capacity of the BC dry film was about 0.116 mg/cm^2 when soaked in 0.102% benzalkonium chloride solution (Fig. 3). As for the antimicrobial activity, stable and prolonged activity was observed for at least 24 h, especially against two Gram-positive bacteria, such as *Staphylococcus aureus* and *Bacillus subtilis*, was generally found on contaminated wounds (Wei, Yang, & Hong, 2011).

BC was formed and coated on cotton gauze samples during its biosynthesis. The composite obtained displayed more than a 30% increase in water absorbency and wicking ability, and a 33% reduction in drying time as compared with untreated gauze (Meftahi et al., 2010). The interactions between BC fibrils and aloe vera gel were investigated by Saibuatong and Phisalaphong (2010). With a 30% (v/v) aloe gel supplement in the culture medium, the fiber-reinforced bio-polymer film obtained displayed significantly improved properties in terms of mechanical strength, crystallinity, water absorption capacity, and water vapor permeability in comparison with unmodified BC films. The average pore size of the modified films in both the dry and re-swollen form was decreased to approximately 20% of the unmodified BC films, while a narrow

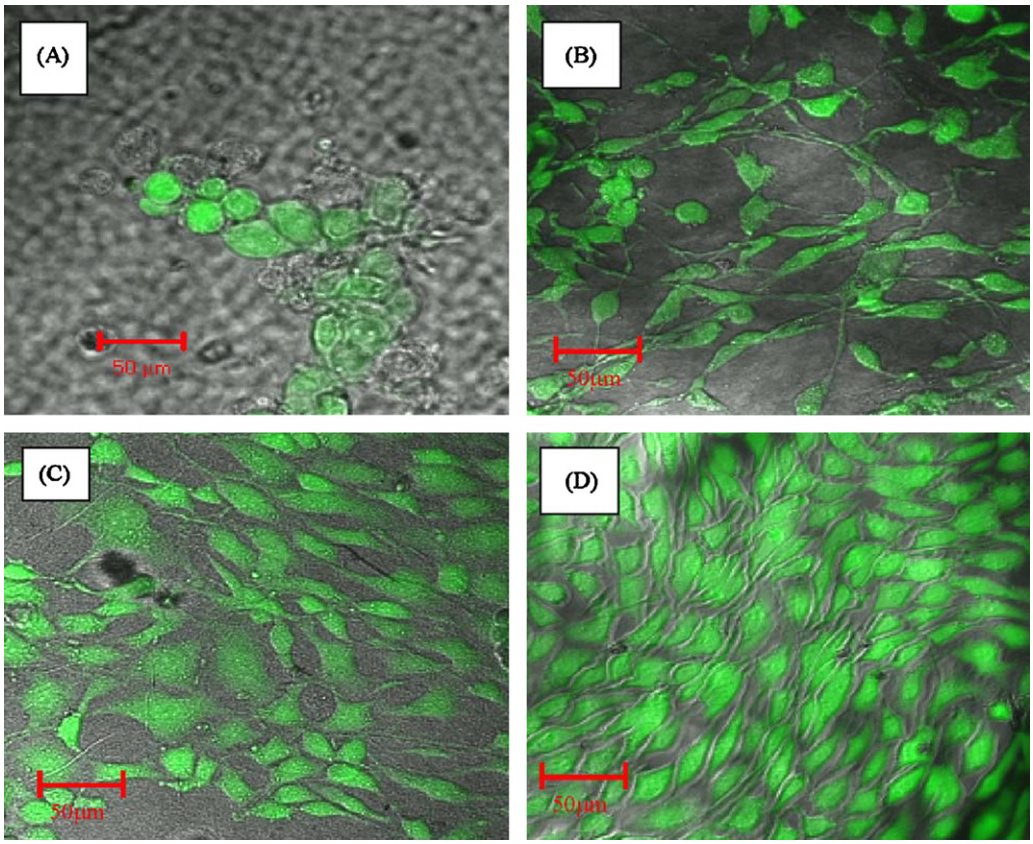

Fig. 2. Confocal laser microscopic images of 6 days old fibroblast NIH/3T3 cells grown on (A) alginate membrane crosslinked with Ca^{2+} (AGM_Ca), (B) BC gel, (C) bacterial cellulose membrane (BC_M) and (D) glutaraldehyde (GTA) crosslinked gelatin membrane, GTA_GM (Nwe et al., 2010).

pore size distribution was maintained (Saibuatong & Phisalaphong, 2010).

BC/poly (ethylene glycol) (PEG) composites were prepared by immersing wet BC pellicle in PEG aqueous solution followed by freeze-drying. SEM images showed that the PEG molecules not only coated the BC fibrils surface, but also penetrated into the BC fiber network. It was found that PEG affected the preferential orientation of the (1–0) plane during the drying of BC pellicle, which in turn decreased the crystallinity of the dried BC film. Thermogravimetric analysis (TGA) results showed that the thermal stability was improved from 263 to 293 °C, which may be associated with strong interactions between BC and PEG (Cai & Kim, 2010). Various BC composites have displayed enhanced applicability as skin tissue repair materials (Table 1).

4.4. Nano-composites of bacterial cellulose and Ag

BC is an optimal material for skin tissue repair since it provides a moist environment for a wound, which is beneficial for healing. Unfortunately, BC itself has no antimicrobial activity to prevent wound infection. To achieve antimicrobial activity, silver nanoparticles and chitosan were combined with BC. Due to the electron-rich oxygen atoms in the BC macromolecules and the large surface area of nanoporous BC effective as nanoreactor, the *in situ* metallization technique was successfully applied to the synthesis of Ag and BC nano-composite, which could in turn serve as antimicrobial skin tissue repair material.

The composite was obtained by immersing BC in a silver nitrate solution, while sodium borohydride was used to reduce the absorbed silver ions (Ag^+) inside BC to metallic silver nanoparticles (Fig. 4). A red-shift and broadening of the optical absorption band was observed. The freeze-dried silver nanoparticle-impregnated BC exhibited strong antimicrobial activity against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) (Maneerung, Tokura, & Rujiravanit, 2008). With the silver nanoparticles absorbed and the N-polyvinylpyrrolidone stabilized, inhomogeneous nanoparticles in the BC gel film were synthesized. The dried composite had large particles located on the

Table 1
BC-based composites for skin tissue repair.

Component	Effect	References
Collagen	Increased the tensile strength, decreased the elongation at break slightly	Cai and Yang (2011)
Gelatin	Enhanced mechanical strength	Nakayama et al. (2004)
Alginate	Changed tensile strength and elongation at break	Phisalaphong et al. (2008), Nwe et al. (2010) and Chiaoprakobkij et al. (2011)
Benzalkonium chloride	Stable and prolonged antimicrobial activity	Wei et al., 2011
PEG	Decreased crystallinity, improved thermal stability	Cai and Kim (2010)
P(3HB-co-4HB)	Increased hydrophilicity and mechanical properties	Cai et al. (2011a)
Cotton gauze	Increased water absorbency, wicking and water retention ability	Meftahi et al. (2010)
Aloe vera	Improved mechanical strength, crystallinity, water sorption capacity, and water vapor permeability	Saibuatong and Phisalaphong (2010)

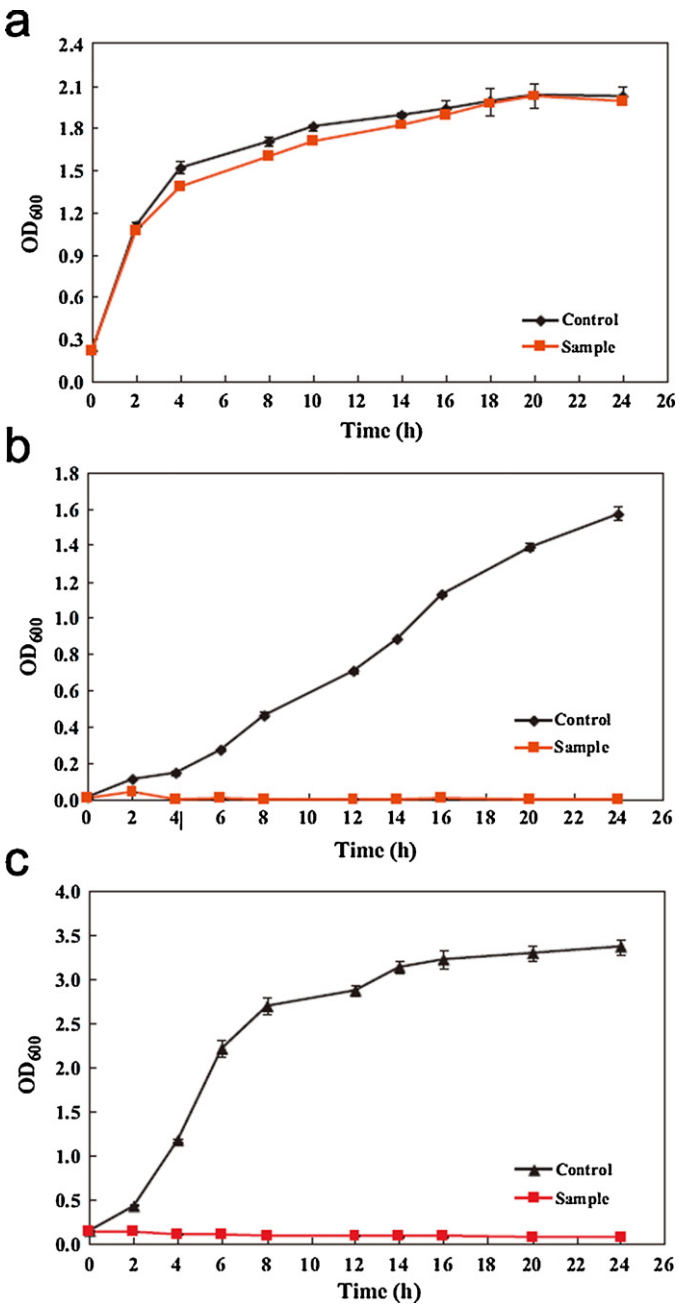

Fig. 3. Comparison of growth curves of three kinds of bacteria cultures with benzalkonium chloride-containing BC dry films and BC without drug as control against (a) *Escherichia coli*, (b) *Staphylococcus aureus*, and (c) *Bacillus subtilis* (Wei et al., 2011).

surface layer of cellulose (Volkov et al., 2009). Colloidal submicron Ag particles were prepared on BC *in situ*. Different reducing agents were compared (hydrazine, hydroxylamine or ascorbic acid) in combination with gelatin or polyvinylpyrrolidone employed as colloid protectors. The Ag cubic phase deposited oil to BC, which resulted in high efficiency of silver loading (Maria et al., 2009).

To obtain a composite of BC and Ag, an ion exchange of sodium to silver salt was performed in an $AgNO_3$ solution, followed by thermal reduction. By using oxidized BC nanofibers as a reaction template, strong ion interactions between the host carboxylate groups from BC and guest silver cations provided stable silver nanoparticles with a narrow size distribution and a high density (Ifuku, Tsuji, Morimoto, Saimoto, & Yano, 2009). The *in situ* synthesis of silver chloride (AgCl) nanoparticles was carried out under ambient conditions by employing nanoporous BC membranes as nanoreactors. Growth of the nanoparticles was readily achieved by alternating the dipping of BC membranes in solutions of silver nitrate and sodium chloride, followed by a rinsing step. The AgCl nanoparticle-impregnated BC membranes exhibited a high hydrophilicity and strong antimicrobial activity against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) (Hu et al., 2009). A simple method was also developed to load a large amount of silver nanoparticles into BC. These composite fibers showed nearly 100% antibacterial activities against *Escherichia coli* (Maria et al., 2010).

A facile method was developed to prepare a magnetic Ag nanocomposite. The 3D nanofibrous structure of BC was first homogenized with a ferric and ferrous salt mixture in a high speed blender. The magnetic BC nanofiber, when soaked in dopamine solution, can be coated with an adherent self-polymerized polydopamine layer. Since the polydopamine surface is very effective for reducing silver ions, Ag nanoparticles were incorporated into the dopamine-treated magnetic BC by soaking in silver nitrate solution. The magnetization of the as-prepared Ag nanocomposite was maintained, and the magnetic Ag nanocomposite possessed high antimicrobial activity against the model microbes *Escherichia coli* and *Bacillus subtilis* (Sureshkumar, Siswanto, & Lee, 2010). BC could also be utilized as the template to *in situ* synthesize silver nanoparticles (AgNPs) through chemical reduction. It was revealed that the particle size of AgNPs the silver content, and the antimicrobial activity of the BC/AgNPs composites varied depending on the different BC templates used, mainly due to the differences in the microstructure of the BC template, especially the crystallinity and porous properties (Yang, Xie, Hong, Cao, & Yang, 2012). Patterned BC might therefore attract more attention in the application of wound healing.

4.5. Nano-composites of bacterial cellulose and chitosan

Nanocomposite films based on different chitosan (CS) matrices (chitosans with two different degrees of polymerization and one water-soluble derivative) and BC were prepared by casting the water-based suspension of chitosan and BC nanofibrils. The films were highly transparent, flexible, and displayed better mechanical properties than the corresponding unfilled chitosan films (Fernandes et al., 2009). The FT-IR analysis results of BC/CS composite showed intermolecular hydrogen bonding interaction between the BC and chitosan molecules. The superior mechanical properties, water holding capacity, and water release rate would thus make the composites suitable for wound dressing and other biomedical applications (Ul-Islam, Shah, Ha, & Park, 2011). A family of polysaccharide-based BC/chitosan porous scaffold materials with various weight ratios (from 20/80 to 60/40 w/w %) were prepared by freezing (−30 and −80 °C) and lyophilization of a mixture of microfibrillated BC suspension and chitosan solution. The microfibrillated BC (MFC) was subjected to 2,2,6,6-tetramethylpyperidine-1-oxyl radical (TEMPO)-mediated oxidation to introduce surface carboxyl groups before mixing. The composite scaffolds had a three-dimensional open pore microstructure with pore sizes ranging from 120 to 280 μm with enhanced compressive moduli and strength (Nge, Nogi, Yano, & Sugiyama, 2010).

By varying the chitosan concentration and immersion time, a foam-like structure was obtained. With increasing chitosan content, the crystalline structure remained unchanged, but the crystallinity index tended to decrease. The tensile strength and Young's modulus of the composites tended to decrease with increasing chitosan content, but the values were much higher than for pure chitosan (Cai, Chen, Jin, & Kim, 2009). Cai, Hou, and Yang (2011b) also investigated the properties of BC/chitosan composite.

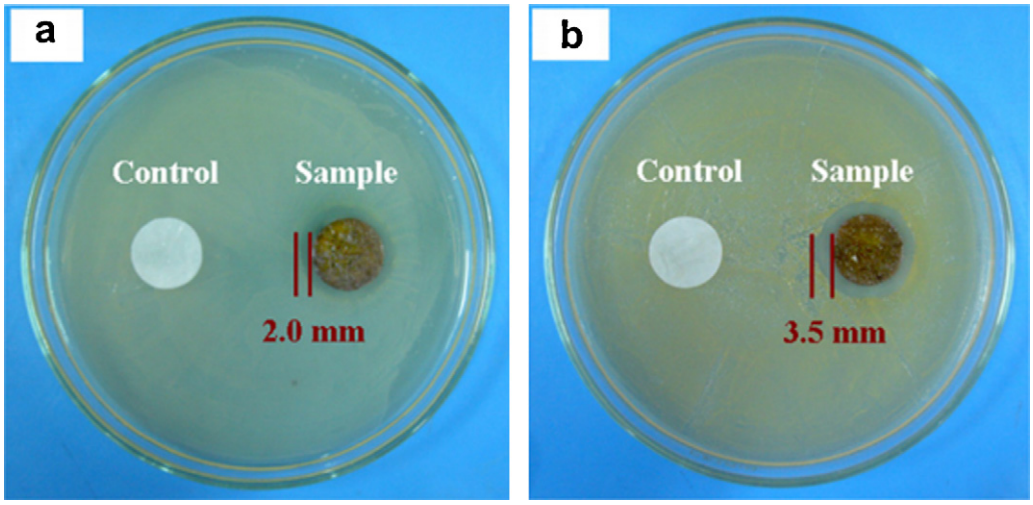

Fig. 4. Antimicrobial activity of freeze-dried silver nanoparticle-impregnated bacterial cellulose prepared from the $NaBH_4$:$AgNO_3$ molar ratio of 100:1 against (a) *Escherichia coli* and (b) *Staphylococcus aureus* (Maneerung et al., 2008).

SEM images showed that chitosan molecules could penetrate into BC, forming three-dimensional multilayer structure scaffold. The crystallinity tended to decrease from 82% to 75% after modification. Moreover, the addition of CS also improved the biocompatibility of BC. BC/chitosan composite has additionally been prepared through the immersion of wet BC in CS solution followed by a freeze-drying process (Kim et al., 2011). SEM images showed that CS molecules could penetrate into BC, forming three-dimensional multilayered scaffold. The scaffold had a very well interconnected porous network structure and large aspect surface. By incorporation of CS into BC, crystallinity tended to decrease from 82% to 61%, while the thermal stability increased.

4.6. Clinical treatment

Following standard care, non-healing lower extremity (LE) ulcers were treated with a BC wound dressing: Dermafill™ (AMD/Ritmed, Tonawanda, NY). The time required for 75% reduction in wound size was then compared for 11 chronic wounds without and with the application of BC. The mean period of observation without the application of BC was 315 days (95% confidence interval (CI): 239–392 days). With the application of BC to these chronic wounds, the mean time for 75% epithelization was reduced to 81 days (95% CI: 50–111 days), with a median value of 79 days. When applied to non-healing LE ulcers, a BC wound dressing clearly shortened the time for wound closure compared with standard care (Portal, Clark, & Levinson, 2009).

The conformability and elastic properties of BC dressing allowed a high degree of adherence to the wound sites, even to moving parts like the torso and face (Fig. 5) and so on. A patient with severe deep second-degree burns of the facial surface was provided with a complete closure of the wound with a single sheet of BC, in which holes for the eyes, nose, and mouth were made after placement. After 44 days, the wounded face was entirely healed with no need for skin grafting and no significant signs of extensive scarring (Czaja, Young, Kawecki, & Brown, 2007). Clinical trials were conducted on 34 patients suffering from severe thermal burns (second-degree A/B) covering 9–18% of the total body surface area (TBSA); 22 of the patients received the BC as testing group. The adherence of BC membrane to the wound surface was excellent in avoiding dead spaces, this can be attributed to its high conformability, while none of the patients using BC wound dressing during the trial developed any kind of hypersensitive reactions. By the tenth day of the treatment period, the process of reepithelization had begun in 7 patients from the testing group (58.3%), in comparison with 4 patients (33.3%) from the control group. These results demonstrated that the application of BC dressing in the treatment of partial thickness burns promoted a favorable environment for fast wound

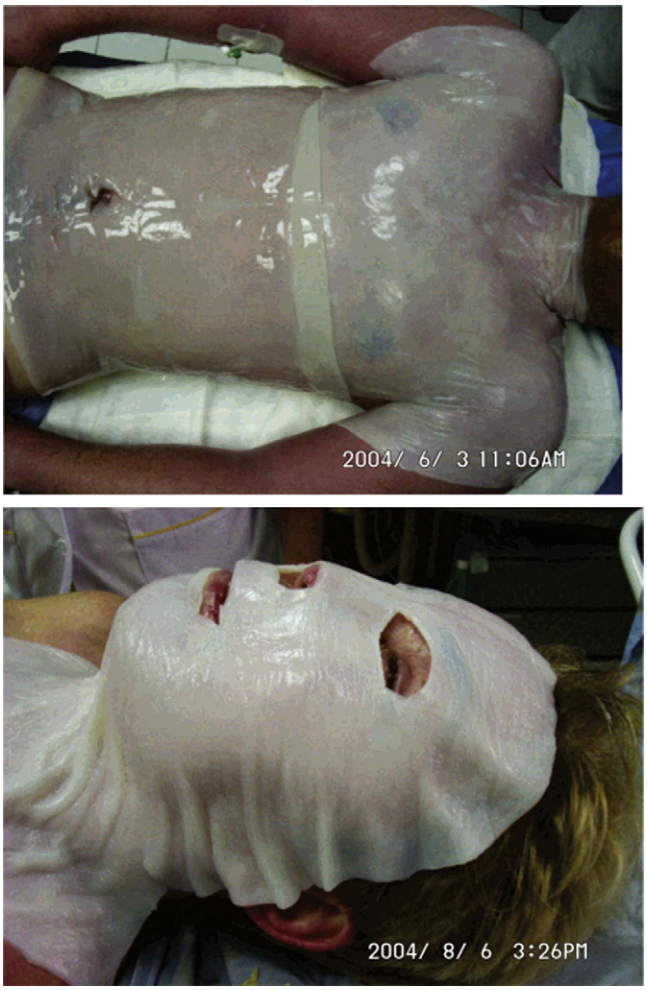

Fig. 5. Bacterial cellulose dressing applied on wounded torso and face. It shows remarkable conformability to the various body contours, maintains a moist environment, and significantly reduces pain (Czaja, Young, et al., 2007).

Table 2
The biomedical applications of BC-based materials.

Applications	Materials	Patents
Skin tissue repair materials	Bacterial cellulose	[ZL 200810047793.7] (Yang, Fu, et al., 2010; Yang, Wang, et al., 2010; Yang, Raczkjowski, et al., 2010)
	Poly(vinyl alcohol)-bacterial cellulose nanocomposite	[US 2005/0037082 A1] (Wan & Millon, 2005)
	Collagen modified bacterial cellulose	[CN 201110300494.1] (Zheng et al., 2012)
	Silver-loaded modified bacterial cellulose	[CN 201110192110.9] (Wang, Zhang, et al., 2011; Wang, Sun, et al., 2011)
	Bacterial cellulose	[US 20110286948 A1] (Lin, Wey, et al., 2011; Lin, Chen, et al., 2011)
	Bacterial Cellulose/hyaluronic acid loading nano-silver composites	[CN 201010139908.2] (Wang, Zhang, et al., 2011; Wang, Sun, et al., 2011)
	Bacterial cellulose loading photocatalytic particles	[US 2009/0209897 A1] (Limaye et al., 2009)
Artificial dura mater	Poly(vinyl alcohol)-bacterial cellulose	[ZL 200710015537.5] (Ma et al., 2010)
	Bacterial cellulose	[CN 201010563139.9] (Liu et al., 2011)
Blood vessels	Carboxymethyl cellulose - bacterial cellulose composite membrane	[CN 200910126692.3] (Cao et al., 2009)
	Bacterial cellulose -heparin composite	[CN 200910067684.6] (Wan et al., 2009)
Bone and connective tissue repair material	Resorbable composites of bacterial cellulose, collagen and hydroxyapatite	[WO 2011150482] (Saska et al., 2011)
	Hydroxyapatite modified bacterial cellulose	[CN 200910036754.1] (Lin & Zhang, 2009)
Biomedical scaffold materials	Bacterial cellulose membrane	[CN 201110191767.3] (Yin et al., 2011)
	Regenerated cellulose and oxidized cellulose membranes	[US 6,800,753] (Kumar, 2004)
Antivirus mask	Bacterial cellulose-nano silver	[ZL 200910149665.8] (Zhong, 2011)
	Bacterial cellulose and silver compounds	[JP 2011167226] (Nakamura & Nakamura, 2011)
	Bacterial cellulose with nanometer silver	[CN 201110191828.6] (Zhang, Wang, Zhou, et al., 2012; Zhang, Wang, Chen, et al., 2012)
	PVA and bacterial cellulose	[CN 201110078333.2] (Zhang, Wang, Zhou, et al., 2012; Zhang, Wang, Chen, et al., 2012)
Make-up product	Bacterial cellulose network, cationic polymer	[US 2011/0039744 A1] (Heath et al., 2011)
	Bacterial cellulose membrane	[ZL 200610075040.8] (Zhong, 2008)
	Bacterial cellulose containing glycerin	[JP 2009077752] (Nakamura & Kawaguchi, 2009)
Cold pack	Bacterial cellulose hydrogel	[ZL 201020239963.4] (Li et al., 2011)

cleansing and rapid healing. It is worth mentioning that the release of the dressing from the wound was an entirely painless operation, due to the moisture still present in the cellulose structure (Czaja, Krystynowicz, et al., 2007).

In a randomized trial on predominantly category II and III skin tears in a population of frail elderly nursing home residents, standard wound care (24 residents) with Xeroform™ and a secondary dressing (Tegaderm™) was compared with a single application of BC Dermafill™ (27 residents). The reported outcomes in the BC Dermafill™ group included a decrease in the time for wound closure, pain reduction, and ease of use. Even though the wound area was slightly larger in the BC-treated group, the healing time was equivalent to the controls. However pain control, ease of use, and patient and nursing staff satisfaction with BC skin tissue repair materials were also superior to the control experiments (Solway, Consalter, & Levinson, 2010). Another test compared the rate of wound healing in diabetic foot ulcers (DFU) using either BC wound dressing or Xeroform™ Petrolatum gauze. 15 ulcers in type II diabetic patients received a BC dressing, while wounds in 19 control patients with type II diabetes were treated with a Xeroform™ gauze dressing. All wounds were non-infected Wagner stage II or III and received standard care including debridement, non-weight bearing limb support, and weekly wound evaluation. With the provision of current care standards, the application of a BC dressing to a diabetic ulcer enhanced the rate of wound healing and shortened the epithelization time (Solway, Clark, & Levinson, 2011). All treatments showed that the use of BC dressings or films was easy to manage, as the patients exhibited a rapid rate of closure with the treatment. The interest in applications of BC has grown rapidly. Therefore, clinical treatment with BC skin tissue repair materials can be considered an efficient method to treat acute and chronic wounds.

5. Patents

Since 1988, more and more patents on the application of BC in various fields have appeared in areas such as the medical material industry, food industry (Yang, Fu, He, Zhou, & Yu, 2010; Yang, Wang, Liu, Shi, & Chen, 2010; Yang, Raczkjowski, Rubic, Mazyck, & Deely, 2010), electronic (Evans et al., 2010), catalytic agent (Patel & Suresh, 2010), photoelectric materials (Hwang et al., 2009), printing materials (Tahara et al., 2000), smart materials (Wu et al., 2011; Yang, Fu, et al., 2010; Yang, Wang, et al., 2010; Yang, Raczkjowski, et al., 2010) and so on.

Owing to the unique nature of BC, its application in medical areas is receiving increased attention, and related patents are increasing. A nano-composite using poly(vinyl alcohol) and BC has previously been prepared; such material can be suitable for a broad range of soft tissue replacement applications (Wan & Millon, 2005). Complexes of the BC and a variety of materials, such as nano-silver (Wang, Zhang, et al., 2011; Wang, Sun, He, & Yang, 2011), collagen (Zheng et al., 2012), and even a BC membrane (Yang, Fu, et al., 2010; Yang, Wang, et al., 2010; Yang, Raczkjowski, et al., 2010) itself can be used as skin tissue repair materials. In addition to soft tissue repair, BC can be used for hard tissue repair and tissue engineering scaffolds. Saska et al. (2011) obtained composites based on BC, collagen, and hydroxyapatite for application in tissue repair in areas such as bone and connective tissue repair (Saska et al., 2011).

Some patents on the applications of bacterial cellulose in medical related fields are presented in Table 2.

6. Conclusion

Bacterial cellulose (BC) is a promising natural polymer with many applications, especially for skin tissue repair. The many

advantages of BC, such as biocompatibility, conformability, elasticity, transparency, ability to maintain a moist environment in the wound and ability to absorb exudate during the inflammatory phase provide great potential for application in wound healing systems. This paper has reviewed the most recent developments in BC-based skin tissue repair materials, including their biosynthesis, methods of treatment, properties, and frontier research on BC skin tissue repair materials. The structure of native and modified BC has been studied intensively and biocompatibility has been evaluated, all of which has suggested that BC can function as a very effective skin tissue repair material.

Materials based on BC have been successfully applied in skin tissue repair and wound dressing fields. In addition, BC can have other applications in wound healing and regenerative medicine, such as guided tissue regeneration, periodontal treatments, or as a replacement for dura mater (the membrane surrounding brain tissue). Moreover, BC is valuable in tissue engineering applications including bone, cartilage, blood vessel engineering, and so on. A large amount of papers and patents of BC have already been reported, demonstrating wide applications. Various modification methods have been applied to improve the properties of BC for skin, bone, and connective tissue repair, and so on. Taken together, it can be assumed that the successful mass-production of BC will eventually lead to it becoming a vital biomaterial for use in a wide variety of medical devices and consumer products, especially in skin tissue repair.

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