13

Bacterial Cellulose for Skin Repair Materials

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1. Introduction

As is well-known, cellulose is one of the most abundant biodegradable materials in nature and has been the topic of extensive investigations in macromolecular chemistry. It is of great importance to explore renewable natural polymeric materials to solve problems such as population growth, the energy crisis, environment polution, etc. Presently, human beings can produce cellulose by four means. Two of these are by natural synthesis procedures including plant photosynthesis and microbial synthesis. The other two methods are synthetic and enzymatic synthesis from cellobiose fluoride *in vitro* and the ring-opening polymerization of new carbonyl derivatives such as nitralin. Over the past 30 years, with the development of molecular biology and the application of cell systems *in vitro*, the mechanism underlying the biosynthesis of cellulose in nature has been extensively explored. Recently, environmental standards have been enhanced for every product and process. Employing new technologies or redesigning products is thus necessary to meet these new environmental standards (El-Saied et al., 2004).

Bacterial cellulose (BC, also known as microbial cellulose, MC) is a promising natural polymer synthesized by certain bacteria. Because of its unique structural and mechanical properties as compared to higher plant cellulose, BC is expected to become a commodity material in various fields. The 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 property, combined with its highly hydrophilic nature, results in a very high liquid loading capacity. Morever, biocompatibility makes it an attractive candidate for a wide range of applications in different 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 never-dry hydrogel state with high strength.

The biosynthetic pathways of BC, including those involving enzymes and precursors, have been described (Chawla et al., 2009). These cellulosic materials are particularly attractive because of their relatively low cost and plentiful supply. Thus, BC utilization is responsible for one of the largest material flows in the biosphere and is of interest in relation to the analysis of carbon flux at both local and global scales (Lynd et al., 2002). Plenty of work has been devoted to designing ideal biomedical devices from BC. Such devices are advantageous in terms of their high paper-like reflectivity, flexibility, contrast, and biodegradability (Iguchi et al., 2000, Klemm, 2006). Besides, BC has proven to be a

remarkably versatile biomaterial and can be used in a wide variety of products such as paper, electronics, acoustics and so on. Cellulose has always been the prime medium for displaying information in our society and is far better than the various existing display technologies. The BC device has the potential to be extended to countless other applications such as e-book tablets, e-newspapers, dynamic wall papers, rewritable maps, and learning tools (Shah, 2005). Olsson et al. used freeze-dried bacterial cellulose nanofibril aerogels as templates to make lightweight porous magnetic aerogels, which can be compacted into a stiff magnetic nanopaper (Olsson et al., 2010). As intuitionistic introduction, the biomedical applications of BC are shown in Figure 1.

However, in most practical applications, BC may not be of perfect quality and its cost may not be suitable for industrialization either. For economical mass production, it is essential to design a culture aeration and agitation process (Yoshinaga, 1997; Yamanaka, 1998). This chapter will discuss the biosynthesis of BC and its application as skin tissue repair material. The skin tissue materials derived from BC may create a luciferous future.

In this chapter, we focus on the applications of BC as skin tissue repair materials. Specifically, we summarize the basic properties, different types of BC, and research on BC for the purpose of applying BC to skin tissue engineering. Experimental results and clinical treatments have demonstrated good performance of BC-based wound healing materials. Further, all the results indicate that BC as skin tissue material in the biomedical field will continue to be important in the future.

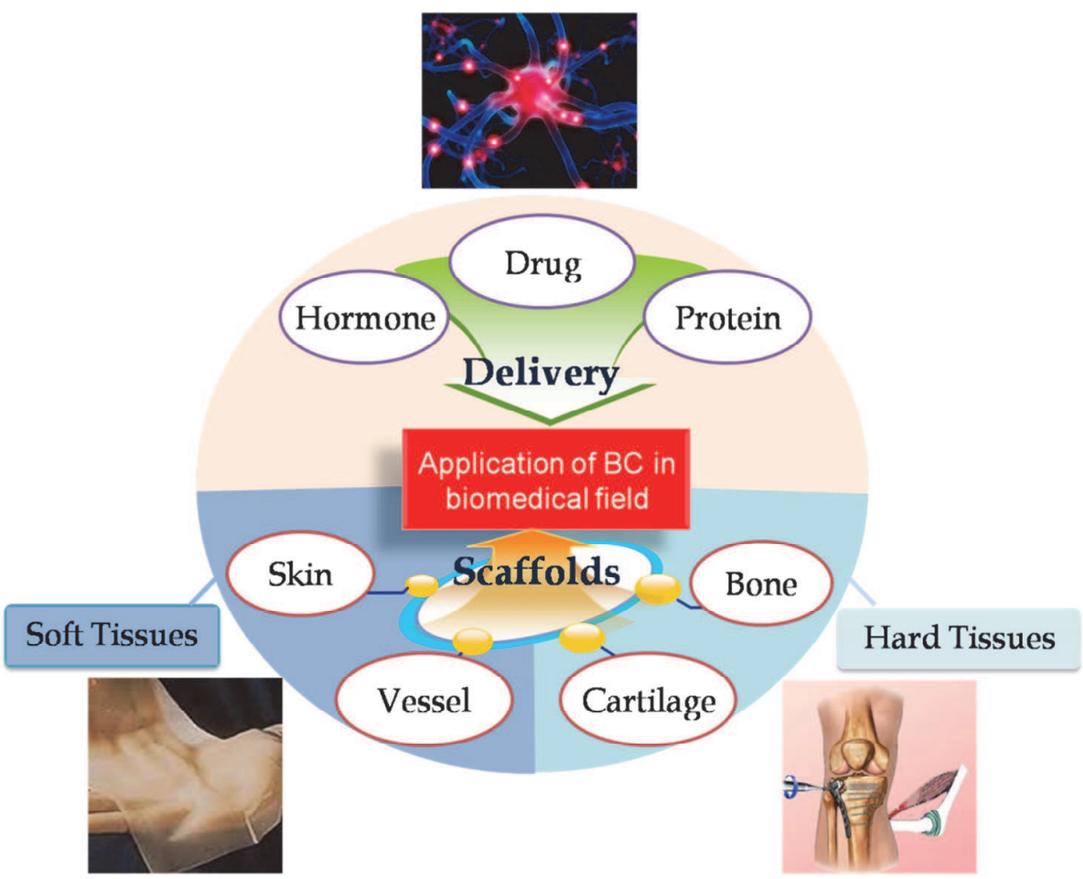

Fig. 1. Biomedical applications of BC-based biomaterials

2. Structure and properties of Bacterial Cellulose

2.1 Structure of BC

Based on X-ray investigations, the orientational state of polymers in general can be defined. BC membranes exhibit uniplanar orientation and an additional axial orientational component that depends on the drying procedure. It is possible to produce uniplanar-axial orientation by drawing, the degree of uniplanar and especially uniaxial orientation depending on the drawing conditions (Bohn et al., 2000). In 1984, VanderHart & Atalla investigated various cellulose samples by Nuclear Magnetic Resonance (NMR) spectroscopy and found that all natural cellulose was a complex of both I_α and I_β forms, and the content of I_α was about 65% in BC (VanderHart & Atalla, 1984). The study of Hirai et al. showed that decreased I_α-cellulose content led to smaller microfibrils in BC (Hirai et al., 1998). Generally, a uniplanar texture with the $(1\bar{1}0)$ planes parallel to the fiber surface and an axial component in the drawing direction were found. As compared to wet aqueous samples, a higher coherent deformation of BC can be achieved by soaking the samples in NaOH solutions with concentration ranging from 8 to 10 wt %. In the presence of lye, significant improvement (up to 100%) in axial chain orientation can be obtained, resulting in a maximum strength of 580 MPa. Improved orientability is likely due to a NaOH-induced reduction in the number of inter-fibrillar bridging points formed by H-bonds (Bohn et al., 2000).

The analysis based on the simple spin diffusion theory for the process experimentally observed reveals that the upfield carbons may be located at a distance less than about 1 nm from the downfield carbons in ^{13}C spin diffusion measurements. It was found that the downfield and upfield carbons were almost equally subjected to ^{1}H spin diffusion from the poly (vinyl alcohol) phase, indicating that the upfield carbons were not localized in some limited area, e.g. in the surfacial region, but were distributed throughout the whole area in the microfibrils. These experimental results suggested that the C4U carbons might exist as structural defects, probably due to conformational irregularity associated with disordered hydrogen bonding of the CH_2OH groups in the microfibrils (Masuda et al., 2003).

2.2 Characterization of bound water

BC is a gel containing 99% of water by weight, mainly due to its amorphous structure. Unfortunately, comparing the water holding capacities of different BC samples is difficult because different methods have been used. Drying under vacuum (10 mm H_2O or 98 Pa) was found to be preferable to stabilize the sample prior to determining its wet weight. This simple method lowered the standard deviation on the measurements by 50% or more as compared to other methods (Schrecker & Gostomski, 2005). According to dielectric spectroscopy and electron microscopy, the majority of the water molecules is tightly bound to BC, while only 10% out of the 99 wt% water presenting in BC gels behaves like free bulk water (Gelin et al., 2007).

The sorption properties of BC gel films were studied by Baklagina et al. The crystal structure of BC remained unchanged when polyvinylpyrrolidone or its complex with silver nanoparticles was incorporated into its matrix. By washing with distilled water, polyvinylpyrrolidone was readily removed from composite gel films of BC and polyvinylpyrrolidone or Poviargol without causing any changes in the cellulose structure and the amount of the adsorbed silver (Baklagina et al., 2005).

2.3 Mechanical properties

Recent studies have shown that atomic force microscopy can be used to measure the elastic modulus of suspended fibers by performing a nanoscale three-point bending test, in which the center of the fiber is deflected by a known force. By calculating the displacement with respect to the applied strain, it was shown that the stiffness of a single fibril of BC could be estimated. To demonstrate this concept, Guhados et al. have measured Young modulus of BC fibers with diameters ranging from 35 to 90 nm at a value of 78 ± 17 Gpa. This value was considerably higher than previous estimates obtained from the mechanical strength of individual cellulose fibers (Guhados et al., 2005). The modulus was also predicted from a calibration curve for a Raman band shift against modulus, based on previously published data, and by using Krenchel analysis to back-calculate the modulus of a single fibril. The value obtained (114 GPa) was higher than those reported previously, but lower than estimates from the modulus of crystalline cellulose-I (130-145 GPa) (Hsieh et al., 2008).

The fermentation time had a large effect on both the number of bacteria and the cellulose yield, but only minor effects on the mechanical properties, indicating that the fermentation technique is a robust method for the production of cellulose with predictable properties. A study by McKenna et al. showed that an increase in the fermentation time could led to a decrease in mechanical strength, Young's modulus first increasing and then decreasing after 96 h. Treatment with NaOH had minimal effects on the mechanical properties. The failure zone in uniaxial tension was shown to be associated with large-scale fibre alignment, this being a major determinant of mechanical properties. As was expected, the elastic modulus and failure stress under uniaxial tension were one order of magnitude lower than the values obtained under biaxial tension, since a fibre alignment mechanism is not available under biaxial tension. BC behaves like a viscoelastic material, brittle failure being reached at approximately 20% strain and 1.5 MPa stress under uniaxial tension (McKenna et al., 2009).

Compression pressure has been found to be an important parameter controlling the final mechanical properties of BC films: 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 higher densification under the increased mold compression pressure which reduced the interfibrillar space, thus increasing the probability of interfibrillar bonding (Retegi et al., 2010).

2.4 Rheology properties

Rheological analysis was developed to evaluate the fibril width and length of disintegrated BC. During the early stage of the disintegration process, the BC particles formed loose fibrous aggregates, followed by cutting of the disintegrated fibrils that produced short fibrils. On the other hand, the fibril width decreased steadily throughout the disintegration process. The relationships between fibril structure and suspension properties were analyzed. The thinner and longer the disintegrated bacterial cellulose fibrils were, the higher the viscosity and water-holding capacity became (Ougiya et al., 1998).

To characterize the mixing of BC culture broth, which can affect the productivity of BC, non-Newtonian behavior during mixing of a 1% BC suspension was studied using an image processor capable of detecting color changes for a pH indicator and was compared with that of a 2% carboxy methyl cellulose (CMC) solution. The CMC solution was mixed homogeneously within the measured range of agitation speeds, while the BC suspension was not homogeneously mixed at agitation speeds lower than 15 rps because mixing was

delayed in some areas of the vessel. A possible reason for the inhomogeneity of the BC suspension at low agitation speeds is the non-Newtonian behavior which increases viscosity at low shear rates (Kouda et al., 1996).

For the three kinds of cellulose solutions, the values of $\eta_0 - \eta_s$ (η_0: zero-shear viscosity of the solution, η_s: solvent viscosity) were in proportion to the weight fraction of polymer, φ_w, in the dilute solution regime. The plateau modulus, G_N, was proportional to φ_w^2 for Cotton linter solutions, signifying that an entangled network structure was formed in the cotton linter solution, as is often observed for solutions of flexible synthetic polymers. On the other hand, the concentrated solution of BC typically displayed small-angle X-ray scattering (SAXS) profiles typical of two-phase systems (Tamai et al., 2003).

3. Bio-fabrication of Bacterial Cellulose

Biodegradable composites made entirely from renewable resources are urgently sought after to improve material recyclability. Many biobased polymers and natural fibers usually display poor interfacial adhesion in composite materials. To modify the surface of natural fibers, BC was utilized as substrates for bacteria during fermentation of BC (Pommet et al., 2008).

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. A structural hydrophobic similar to the "Lotus effect" on this sheet was thus examined by introducing a micro-lattice pattern onto 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 et al., 2009).

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).

Since the oxygen produced by the electrolysis of water in the culture media is far from the liquid-air boundary, aerobic cellulose production into 3D structures is readily achievable. Five separate sets of experiments were conducted to demonstrate the assembly of nanocellulose by *A. xylinum* (*G. 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 et al., 2010).

The effect of agar plates on BC production in a static culture medium was investigated in order to reveal the role of the agar component as a surface-modifying agent. 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 et al., 2010).

BC with an unoriented microfibril network forms at the air-liquid interface (BC-air), while BC gel can be produced on an oxygen-permeable substrate such as polydimethylsiloxane (PDMS). The gel thus obtained shows strong birefringence with colorful images in polarized light microscopy, which is typical of liquid crystal-like structures. 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 contents from about 1.8 μm (1 wt %) to about 100 nm (20 wt %). The nominal content of cellulose in the fibers was assessed by Lorentzian profile fit assignment of the crystalline phase, and the results showed significantly improved thermal stability for the composite material. The fibers were aligned into an anisotropic nanocomposite during spinning (Olsson et al., 2010).

3.2 Magnetic Bacterial Cellulose

Uniform magnetic membranes can be obtained from microfibrillar bacterial cellulose suspensions loaded with nanosized ferrites (mainly magnetite). The cellulose microfibrils act as a nucleation site for the growing ferrites (Sourty et al., 1998). Ferrites were thus synthetized in situ in two different neutral cellulose gels: a never-dried bacterial cellulose membrane and a never-dried film cast from N-methylmorpholine-N-oxide. The results showed the presence of ferrites in two different shapes, acicular and equiaxial, respectively corresponding to hydrated ferric oxides (FeOOH) and the spinel oxides (maghemite, γ-Fe_2O_3, or magnetite, Fe_3O_4). Thin sections of bacterial cellulose showed that these particles were located along the cellulose microfibrils, which were assumed to provide sites for the nucleation of these particles. Room temperature magnetization curves showed that all the samples were superparamagnetic (Sourty et al., 1998). Bacterial cellulose, with its porous network structure, was also used as an accelerator to precipitate Ni nanoparticles by 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 et al., 2010).

3.3 Modification of Bacterial Cellulose

The process of modifying large quantities of natural fibers with BC was investigated, and the adhesion between the modified fibers and renewable polymers such as cellulose acetate butyrate and poly(L-lactic acid) was quantified by employing the single fiber pullout test (Bodin, 2010), providing new ideas for the modification of BC. Natural fibers have been modified for the reinforcement of polymers, for example 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. 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).

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 at modifying the surface of BC nanofibrils and improving the compatibility of PLA/cellulose composites (Li et al., 2010). Moreover, polylactide-graft-methacryloxypropyltrimethoxysilane (PLA-g-MPS) was prepared by grafting MPS onto PLA, and then used to modify BC. The results revealed

that the modified BC possessed a much more hydrophobic nature than virgin BC (Li et al., 2010).

3.4 Multiform Bacterial Cellulose

The field of application of BC synthesized by *A. xylinum* under agitated culture conditions is narrower than for cellulose produced statically. This is mainly due to the smaller crystallite size of the microfibrils produced in agitated cultures. A mechanism was proposed to explain BC sphere formation from the microfibrils and cell arrangement in agitated cultures. During agitation, the cells were stacked in organized groups around the outer surface of the cellulose spheres (Czaja et al., 2004). Spherelike BC formaion has been investigated as a function of agitation speed and flask size. The analysis of lyophilized spherelike cellulose particles indicated that the agitation speed of the culture had an impact on the internal structure of the spherelike particles. The smaller spherelike particles produced at 150 rpm were hollow and their cellulose shell exhibited a layered structure. The larger particles produced at 125 rpm, and the cellulose in the central region did not exhibit a layered structure, while the outer layer was similar in structure to the particles produced at 150 rpm (Hu et al., 2010).

Phase separation phenomena in aqueous suspensions of BC nanocrystals obtained by sulfuric acid hydrolysis have been studied. Suspensions at concentration 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 incvreased, reaching a minimum value at approximately 0.75 mM, and then increased sharply with the NaCl concentration up to 2.0 mM (Hirai et al., 2009). Obtaining a well-dispersed suspension is a prerequisite when preparing smooth model surfaces based on neutral bacterial cellulose nanocrystals (BCNs). However, neutral nanocrystal suspensions suffer from pronounced particle aggregation. Carboxymethyl cellulose (CMC) or xyloglucan (XG) were added to the aggregated BCN 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 obtained 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 reference unmodified BCN model surface (Winter et al., 2010).

4. Skin tissue repair materials from Bacterial Cellulose

Owing to its unique nano-scaled three-dimensional network structure, BC has a high water retention, high mechanical strength, and outstanding biocompatibility, which enable it to serve as a natural scaffold material for the regeneration of a wide variety of tissues (MacNeil, 2007; Siró, 2010; Klemm, 2006; Czaja, 2006). For most repair materials, important characteristic are their ability to lock exudate during the dressing process, as well as 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 induce trauma on removal of the dressing. Recently, interest in cellulose produced by bacteria from surface cultures has

increased steadily because of its potential for application in medicine and cosmetics (Hornung et al., 2009). On one hand, its potential lies in the unique properties (such as the high mechanical strength) of the never-dried BC membrane; on the other hand, its high liquid absorbency, biocompatibility and hygienic nature perfectly cater to the specific demands for skin tissue repairing. Thus, considering the properties of BC as well as its clinical performance, the commercialization of BC for wound care is very promising (Czaja et al., 2006).

4.1 Basic properties of skin tissue repair materials

Compared to plant cellulose, BC has features such as a high crystallinity, tensile strength and water absorption capacity; good permeability; biocompatibility; resistance to degradation and a low solubility that may be advantageous features 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). The freezable bound water behaves like water confined within pores rather than a typical polymer solvent, and it is possible to use the Gibbs-Thomson equation based on thermoporosimetry to obtain information on the pore structure of BC. In comparison with nitrogen adsorption, it was found that thermoporosimetry underestimated the porosity of BC, which may be due to a large non-freezable water fraction interacting with cellulose (Kaewnopparat et al., 2008).

The water vapor permeability of air-dried BC is quite excellent because of the presence of a large number of hydroxyl groups. BC membranes are highly selective to water; the highest selectivity observed [$\alpha(p) = 186$] was obtained for a mixture of trihydric alcohol viz. glycerol (Gly) with 40% (v/v) water. The binary system of monohydric alcohol viz. ethanol (EtOH) and water (40% (v/v)) showed the lowest selectivity [$\alpha(p) = 12$] but the highest pervaporative flux of 614 $g \cdot m^{-2} \cdot h$ at 35 ^{o}C, which further increased to 1429 $g \cdot m^{-2} \cdot h$ at 75 ^{o}C. However, the selectivity also decreased to 1.3 with the increase in temperature. The pervaporation behaviour was interpreted in terms of sorption and diffusivity of the organics, which in turn was influenced by the extent of their hydrogen bonding with the cellulose units in the membrane and the plasticization induced by the permeating water present in the binary mixture (Pandey et al., 2005).

4.2 Biocompatibility of skin tissue repair materials

BC is 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).

Helenius et al. implanted BC subcutaneously into rats and evaluated the implants with respect to chronic inflammation, foreign body responses, cell ingrowth, and angiogenesis through histology, immunohistochemistry, and electron microscopy. There were no macroscopic signs of inflammation around the implants: No fibrotic capsule or giant cells were present. Fibroblasts infiltrated BC, which was well integrated into the host tissue and did not elicit any chronic inflammatory reactions (Helenius et al., 2006). The *in vitro* evaluation of the interactions between cells and BC was performed through viability staining analysis on the cells grown on the biomaterial, and showed that 95% of the mesenchymal stem cells aggregating to the cellulose membrane were alive and that 5% were necrotic. Scanning electron microscopy showed that mesenchymal stem cells were morphologically normal and attached to the cellulose membrane surface (Mendes et al., 2009).

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. To improve the cell biocompatibility of BC, Andrade et al. grafted RGD onto BC films that exhibited improved biocompatibility (Andrade et al., 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 demonstrated by Pertile et al. (Pertile et al., 2010). Specially, microporous BC scaffolds were seeded with urine-derived stem cells, which were induced to differentiate into urothelial and smooth muscle cells (Bodin, 2010).

4.3 Composites of Bacterial Cellulose

While BC can be used as skin tissue repair material, it has no significant influence on the biochemical state of chronic wounds. To improve the positive features of BC as wound dressing material, it was modified by the incorporation of collagen type I into a cellulose pellicle. The modified biomaterial was able to reduce the adsorbed amounts of certainproteases and interleukins significantly and possessed a distinct antioxidant capacity as well (Wiegand et al., 2006).

Double-network (DN) hydrogels with high mechanical strength were synthesized from BC and gelatin. The fracture strength and elastic modulus of a BC-gelatin DN gel under compressive stress were on the order of megapascals, which is several orders of magnitude higher than for a gelatin gel, and almost equivalent to articular cartilage. Similar enhancement in the mechanical strength was also observed for a combination of BC with polysaccharides such as sodium alginate, gellan gum, and i-carrageenan (Nakayama et al., 2004). For example, the membrane 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 et al., 2008). Beside the composite with alginate, BC and gelatin were also selected to prepare membranes and the morphology of Swiss mouse embryo fibroblast 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. Membranes derived cow bone gelatin and fish skin gelatin were stronger and more flexible than those prepared from pork skin gelatin in their wet forms (New et al., 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 showed that the drug-loading capacity of the BC dry film was about 0.116 mg/cm² when soaked in 0.102% benzalkonium chloride solution (Fig. 2). As to the antimicrobial activity, a stable and prolonged activity was observed for at least 24 h, especially against *Staphylococcus aureus* and *Bacillus subtilis*, two Gram-positive bacteria generally found on contaminated wounds (Wei et al., 2011).

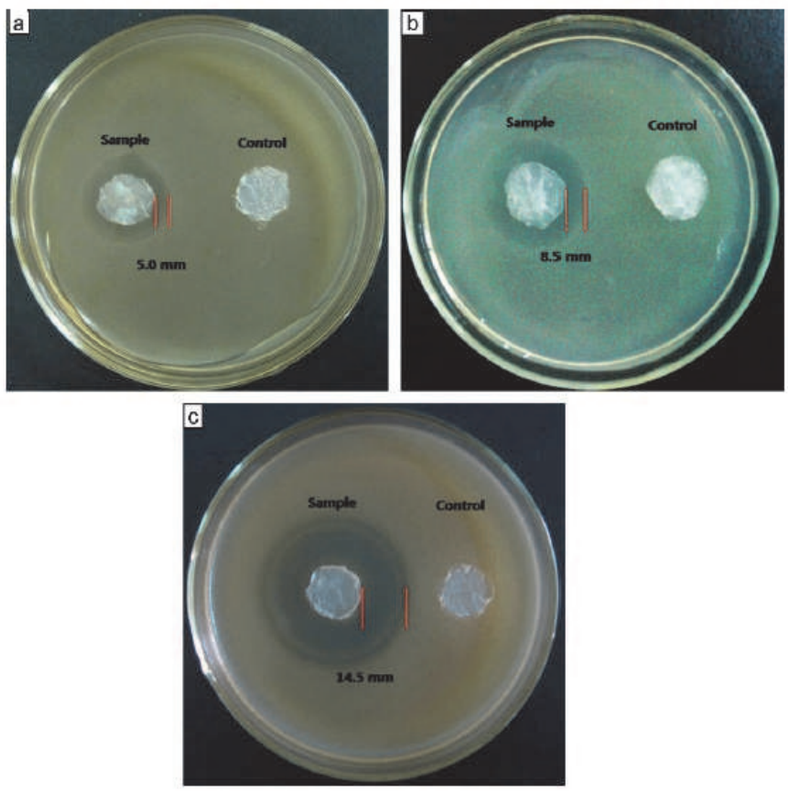

Fig. 2. Comparison of the antibacterial activity of benzalkonium chloride-containing BC dry films and BC without drug as control against (a) *Escherichia coli*, (b) *Staphylococcus aureus*, and (c) *Bacillus subtilis*. (Reproduced with the permission from Wei, B. et al. (2011). Preparation and evaluation of a kind of bacterial cellulose dry films with antibacterial properties, Carbohydrate Polymers, Vol. 84, No.1, pp.536. Copyright (2011) Elsevier)

BC was formed and coated on cotton gauze samples during its biosynthesis. The composite obtained displayed more than 30% increase in water absorbency and wicking ability, and a 33% reduction in drying time as compared to untreated gauze (Meftahi et al., 2010).
The interactions between BC fibrils and aloe vera gel were investigated by Saibuatong et al. With a 30% v/v aloe gel supplement in the culture medium, the fibre-reinforced bio-polymer film obtained displayed significantly improved properties in terms of mechanical strength, crystallinity, water absorption capacity, and water vapor permeability in comparison to unmodified BC films. The average pore size of the modified films either in the dry or re-swollen form was decreased to approximately 1/5 of the unmodified BC films, while a narrow pore size distribution was maintained (Saibuatong et al., 2010).

BC/poly (ethylene glycol) (PEG) composites were prepared by immersing wet BC pellicle in PEG aqueous solutions followed by freeze-drying. Scanning electron microscope (SEM) images showed that the PEG molecules not only coated on the BC fibrils surface but also penetrated into the BC fiber network. It was found that PEG affected the preferential orientation of the $(1\bar{1}0)$ plane during drying of the 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 degrees C, which may be associated with strong interactions between BC and PEG. Biocompatibility of the composite was preliminarily evaluated by cell adhesion studies using 3T3 fibroblast cells. Incubation of the cells with the BC/PEG scaffolds accelerated cell adhesion and proliferation (Cai et al., 2010). Various BC composites have displayed enhanced applicability as skin tissue repair materials (Table 1).

Component	Effect	References
Collagen	Reduced sorption of proteases and interleukins	Wiegand et al., 2006
DN gelatin hydrogels	Enhanced mechanical strength	Nakayama et al., 2004
Alginate	Changed tensile strength and elongation at break	Phisalaphong et al., 2008; New et al., 2010
Benzalkonium chloride	Stable and prolonged antimicrobial activity	Wei et al., 2011
PEG	Decreased crystallinity, improved thermal stability	Cai et al., 2010
Cotton gauze	Increased water absorbency, wicking and water retention ability	Meftahi et al., 2010
Aloe vera gel	Improved mechanical strength, crystallinity, water sorption capacity, and water vapor permeability	Saibuatong et al., 2010

Table 1. Composites of Bacterial cellulose

4.4 Nano-composites of Bacterial Cellulose and Ag

BC is an optimal material for skin tissue repair since it provides a moist environment to a wound, which is beneficial to 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-composites, which could serve as antimicrobial skin tissue repair materials.

The composite was obtained by immersing BC in a silver nitrate solution, and sodium borohydride was used to reduce the absorbed silver ions (Ag^+) inside of BC to metallic silver nanoparticles (Fig.3). 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 et al., 2008).

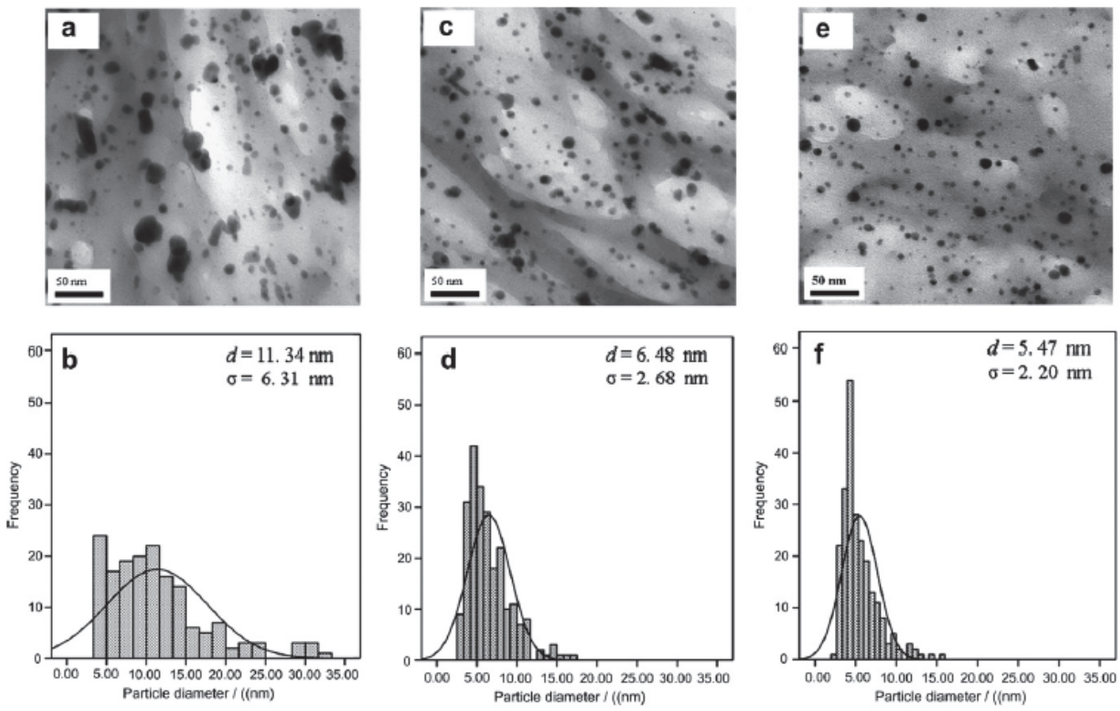

Fig. 3. TEM images and histograms of freeze-dried silver nanoparticle-impregnated bacterial cellulose prepared from a $NaBH_4$:$AgNO_3$ molar ratio of 1:1 (a and b), 10:1 (c and d) and 100:1 (e and f) (Reproduced with the permission from Maneerung, T et al. (2008) . Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing, Carbohydrate Polymers, Vol.72 , No. 1, pp. 48. Copyright (2008) Elsevier)

With absorbed silver nanoparticles and stabilized by N-polyvinylpyrrolidone, inhomogeneous nanoparticle in the BC gel film were synthesized. The dried composite had large particles located on the layer surface 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 a high efficiency of silver loading (Maria et al., 2009).

To obtain the composite of BC and Ag, an ion exchange of the sodium to the silver salt was performed in an $AgNO_3$ solution, followed by thermal reduction. By using oxidized BC nanofibers as a reaction template, stable silver nanoparticles were prepared with a narrow size distribution and a high density, through strong ion interactions between the host carboxylate groups from BC and guest silver cations (Ifuku et al., 2009). The *in situ* synthesis of silver chloride (AgCl) nanoparticles was carried out under ambient condition by employing nanoporous BC membranes as nanoreactors. Growth of the nanoparticles was readily achieved by alternating 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 on a high speed blender. The magnetite nanoparticles were precipitated and incorporated into the BC nanostructures by adjusting the homogenate to alkaline pH. 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 the silver ion, 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 a high antimicrobial activity against the model microbes *Escherichia coli* and *Bacillus subtilis* (Sureshkumar et al., 2010).

4.5 Nano-composite of Bacterial Cellulose and chitosan

Nanocomposite films based on different chitosan 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, which is a fully "green" procedure. The films were highly transparent, flexible, and displayed better mechanical properties than the corresponding unfilled chitosan films (Fernandes et al., 2009). BC/chitosan composite materials showed a high sensitivity to enzymatic degradation and bioactivity. This innovatively modified BC nevertheless represents a good value for biomedical applications (Ciechanska, 2004). The potential of chitosan/BC was compared with that of the parent polymers and with chitosan/poly(vinyl alcohol) blends (Dubey et al., 2005).

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 et al., 2009). 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 degrees 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 et al., 2010).

4.6 Clinical treatment

Following standard care, nonhealing 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 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% 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 nonhealing LE ulcers, a BC wound dressing clearly shortens the time to wound closure over standard care (Portal et al., 2009).

Clinical trials were conducted on 34 patients suffered 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 to avoid any dead spaces because of its high conformability, and 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 reepithelialization had begun in 7 patients from the testing group (58.3%) in comparison with 4 patients (33.3%) from the control group. These results show that the application of BC dressing in the treatment of partial thickness burns promotes the creation of a favorable environment for fast wound cleansing, and consequently its 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 never-dried cellulose structure (Czaja et al., 2007) .

The conformability and elastic properties of BC dressing allowed a high degree of adherence to the wound sites, even to the moving parts like hands (Fig. 4), torso, faces (Fig. 5) and so on. A complete closure of the wounded face with a single sheet of BC in which the holes for eyes, nose, and mouth were made after placement has been applied to a patient with the severe deep second-degree burns of the facial surface. After 44 days, the wounded face was entirely healed with no need for skin grafting and no significant signs of extensive scarring (Czaja et al., 2007).

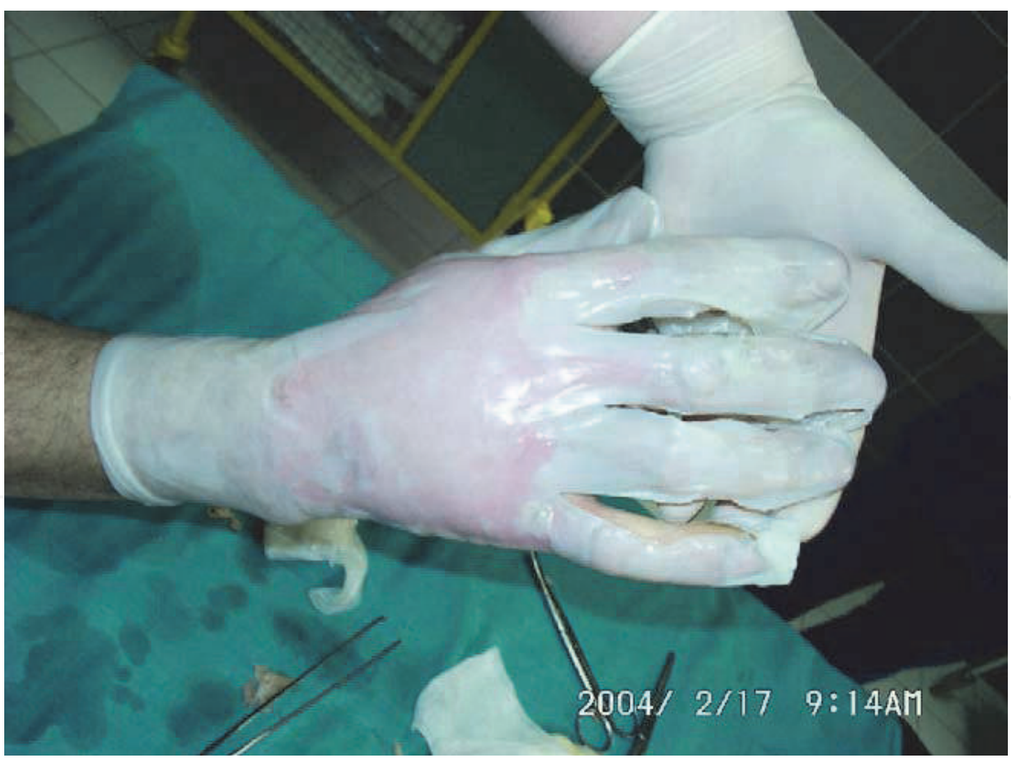

Fig. 4. Bacterial cellulose dressing applied on a wounded hand. (Reproduced with the permission from Czaja, W. et al . (2006). Microbial cellulose — the natural power to heal wounds, Biomaterials, Vol.27, No. 2, 149. Copyright (2006) Elsevier)

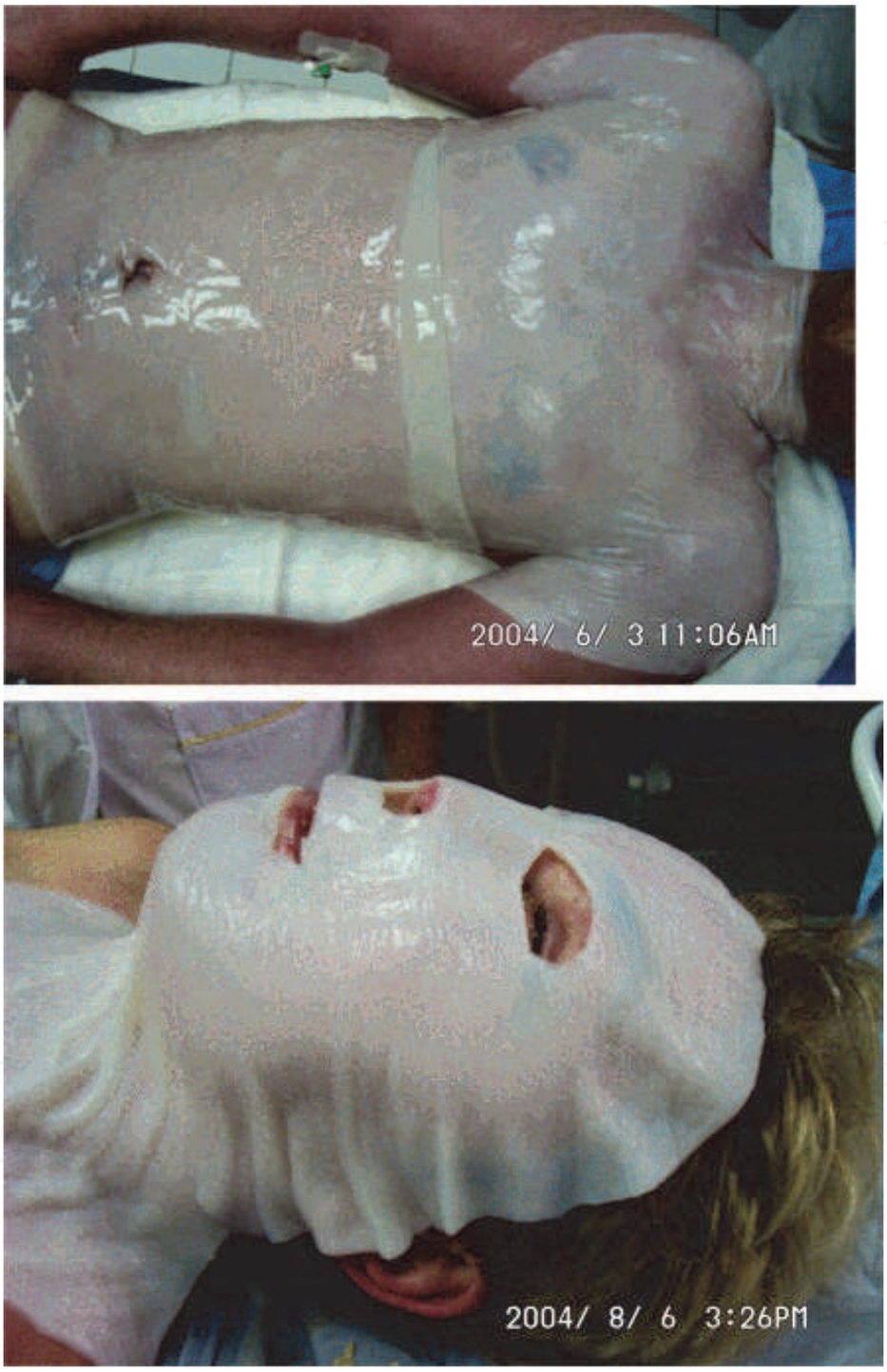

Fig. 5. Bacterial cellulose dressing applied on wounded torso and face. (Reprinted with permission from Czaja, W. K. et al. (2007). The future prospects of microbial cellulose in biomedical applications, Biomacromolecules, Vol.8, No.1, pp. 4. Copyright (2007) American Chemical Society)

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). Outcomes included a decrease in the time to 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 were superior to the control experiments with the use of the BC skin tissue repair materials (Solway et al., 2010). Another test compared the rate of wound healing in diabetic foot ulcers (DFU) using either BC wound dressing or Xeroform™ Petrolatum gauze. In a parallel, open-label trial in which the primary outcome was the rate of wound healing and the time to wound closure, 15 ulcers in type II diabetic patients received a BC dressing. 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 epithelisation time (Solway et al., 2011). All treatments showed that using BC dressings or films was easy to manage because the patients exhibited a rapid rate of closure with the treatment. 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, the interest in applications of BC has grown rapidly (Bielecki et al., 2005). Some patents concerning different aspects are presented in Table 2.

Material	Applications	Patents
Bacterial cellulose hydrogel	cold pack	[ZL 201020239963.4] (Li et al, 2011)
Bacterial cellulose-nano silver	Mask	[ZL 200910149665.8] (Zhong, 2011)
bacterial cellulose membrane	Membrane electrode	[ZL 200810022130.X] (Xu et al, 2011)
Metalized bacterial cellulose	Construction of fuel cells, electronic devices	[US 7,803,477 B2] (Evans et al., 2010), [US 2011 / 0014525 A1] [85] (Evans et al., 2011)
Bacterial cellulose network, cationic polymer	Personal cleansing compositions	[US 2011 / 0039744 A1] (Heath et al., 2011)
Bacterial cellulose	Cultural relics conservation	[ZL 200810246345.X] (Wu et al, 2011)
Bacterial cellulose	Skin tissue repair materials	[ZL 200810047793.7] (Yang et al., 2010)

Patterned bacterial cellulose	Smart materials	[ZL 200810047875.1] (Yang et al., 2010)
Novel bacterial cellulose	Food industry	[US 2010 / 0016575 A1] (Yang et al., 2010)
Poly(vinyl alcohol)- bacterial cellulose	Artificial dura mater	[ZL 200710015537.5] (Ma et al, 2010)
Palladized bacterial cellulose	Reductive conversion reactions	[US 2010 / 0126945 A1] (Patel & Suresh, 2010)
Bacterial cellulose network	Liquid detergent composition	[US 2010 / 0210501 A1] (Caggioni et al., 2010)
modified bacterium cellulose	Food wrap	[ZL 200810051298.3] (Yu et al, 2010)
Bacterial cellulose	Carbon nanotube-like thin films, cathode material, batteries	[US 2009 / 0309072 A1] (Hwang et al., 2009)
bacterial cellulose membrane	face mask	[ZL 200610075040.8] (Zhong, 2008)
Bacterial cellulose	Viscosity modifier	[US 2007 / 0197779 A1] (Yang et al., 2007)
Novel bacterial cellulose	Viscosity modifier	[US 2007 / 0027108 A1] (Yang et al., 2007)
Poly(vinyl alcohol)- bacterial cellulose nanocomposite	Soft tissue replacement, medical devices	[US 2005 / 0037082 A1] (Wan. & Millon, 2005)
Bacterial cellulose	Industry, clothes, medical supplies, food, functional materials	[US RE38,792 E] (Iguchi et al., 1988), [US 2004/ 0091978 A1] (Ishihara & Yamanaka, 2004), [US 2002/ 0065409 A1] (Ishihara & Yamanaka, 2002)
Bacterial cellulose	Yield improvement in BC production	[6,132,998] (Naritomi et al., 2000)
Bacterial cellulose	Improvement of the properties of paper	[6,069,136] (Tahara et al., 2000)
Bacterial cellulose	In creased BC production rate and yield	[6,017,740] (Kouda et al., 2000)
Bacterial cellulose	Increased BC production rate	[6,013,490] (Kouda et al., 2000)
Enzymatic detergent drain cleaners	Removal or prevention of BC growth	[5,975,095] (Ahmed et al., 1999)
Gelationous bacterial cellulose	Production of soft and light fibers	[5,962,676] (Tammarate, 1999)
Reticulated bacterial cellulose	Coating on a substantially continuous basis, coated products	[5,637,197] (Watt et al., 1997)

Reticulated bacterial cellulose	Reinforced elastomeric articles, pneumatic tires	[5,290,830] (Tung et al., 1994)
Bacterial cellulose	Banding agent	[5,207,826] (Westland et al., 1993)
Purified bacterial cellulose	Binding- suspended cholesterol or cholesterol esters	[4,960,763] (Stephen et al., 1990)
Bacterial cellulose	Replacement for latex binders	[4,919,753] (Johnson & Neon, 1990)
Bacterial cellulose	Printing materials	[4,861,427] (Johnson et al., 1989)
Bacterial cellulose	Molding material	[4,742,164](Iguchi et al., 1988)

Table 2. Applications of Bacterial Cellulose

6. Conclusions

Bacterial cellulose (BC) is a promising natural polymer with many applications, especially for skin tissue repairing. Many advantages of BC give it great potential in wound healing system, such as biocompatible, conformability, elasticity, transparency, ability to maintain a moist environment in the wound and absorb exudates during inflammatory phase, and so on. This chapter discussed 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 having been studied intensively and biocompatibility having been evaluated, suggested that BC could function as a skin tissue repair material well. Different BC products having been successfully applied as skin tissue repair and wound dressing materials, confirmed this. In addition, BC could 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). Last but not least, BC is valuable in tissue engineering applications including bone, cartilage, blood vessel engineering, and so on. In a conclusion, if BC can be successfully mass-produced, it will eventually become a vital biomaterial used in the creation of a wide variety of medical devices and consumer products.

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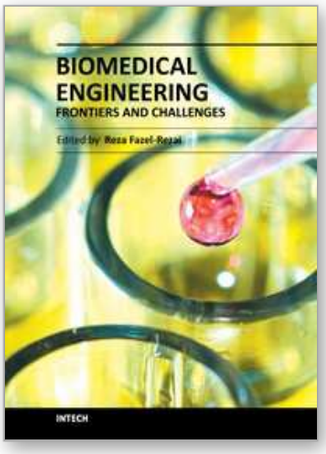

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In all different areas in biomedical engineering, the ultimate objectives in research and education are to improve the quality life, reduce the impact of disease on the everyday life of individuals, and provide an appropriate infrastructure to promote and enhance the interaction of biomedical engineering researchers. This book is prepared in two volumes to introduce recent advances in different areas of biomedical engineering such as biomaterials, cellular engineering, biomedical devices, nanotechnology, and biomechanics. It is hoped that both of the volumes will bring more awareness about the biomedical engineering field and help in completing or establishing new research areas in biomedical engineering.

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