Bacterial cellulose as a material for wound treatment: Properties and modifications. A Review

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Research review paper

Bacterial cellulose as a material for wound treatment: Properties and modifications. A review

Irina Sulaeva, Ute Henniges, Thomas Rosenau, Antje Potthast *

University of Natural Resources and Life Sciences, Department of Chemistry, Division of Chemistry of Renewable Resourses, Konrad-Lorenz Straße 24, 3430 Tulln an der Donau, Austria

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ABSTRACT

Advanced approaches to wound healing have attracted much attention in the last decades due to the use of novel types of dressings that provide a moist environment and take an active part in wound protection and tissue regeneration processes. The materials for novel wound dressings should have a set of features that will contribute to efficient skin recovery. The use of bacterial cellulose (BC) is attractive for advanced wound management because of the favorable characteristics of BC, such as its biocompatibility, non-toxicity, mechanical stability, and high moisture content. Numerous approaches can be taken to modify BC to address the shortcomings of the native material and to optimize its biocompatibility, water uptake and release, and antimicrobial activity. This review highlights possible pathways for functionalization of BC, affecting all levels of its structural organization. The focus is on post-production treatment of BC, although selected studies concerning *in situ* modifications during the biosynthesis process are also emphasized.

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* Corresponding author.
E-mail addresses: thomas.rosenau@boku.ac.at (T. Rosenau), antje.potthast@boku.ac.at (A. Potthast).

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1. Introduction

People have used wound dressings for treatment of severe skin burns and injuries for centuries. Historically, the principal role of a dressing in the healing process was considered to be passive protection of the wound. The primary function of traditional gauze-based dressings, such as woven and non-woven sponges as well as natural and synthetic bandages was to keep the wound dry. Exudate absorption and evaporation, together with prevention of bacterial invasion, were believed to play a key role in successful wound healing. This view on wound management, however, has been changing significantly over the last few decades. A dressing is no longer considered a passive supplement, but an active component of the healing process that is designed to control infection and provide a propitious healing microenvironment. A warm, moist environment is now recognized as one that encourages fast and effective healing, and is particularly important when dealing with chronic wounds (Bergstrom et al., 2005; Lee et al., 2009).

The global market currently offers different types of wound dressings for advanced wound management based on various materials—including natural or synthetic polymers, as well as their combinations. Implemented in different forms (films, foams, hydrocolloids, and hydrogels), these materials may contain drugs, growth factors, peptides, and other bioactive substances that can accelerate recovery (Bergstrom et al., 2005). The actual requirements of an "ideal dressing" are quite demanding: it must provide a moist environment, thermal insulation, and effective oxygen circulation; ensure liquid drainage and epithelial migration; aid in absorption of wound exudates; provide wound protection from bacterial loads and secondary infections; it must be easy to apply and painless to remove; and it should be biocompatible without provoking allergic reactions (Fonder et al., 2008; Watson and Hodgkin, 2005). This diversity of desirable characteristics imposed on modern wound healing devices is summarized in Fig. 1. These individual physicochemical characteristics of a dressing may alter wound healing, but the specific and complex process of wound recovery is affected by many other factors, such as the type of wound being treated (e.g., acute, chronic, exuding, or dry wounds, etc.), patient health conditions (the presence of other diseases, e.g., diabetes, anemia), and the social environment. Therefore, the selection of an appropriate dressing is determined by the particularity of every individual occurrence, since none of the currently existing materials is able to fulfill all the requirements of an "ideal dressing" (Lagana and Anderson, 2010). Among different dressing materials, hydrogels are currently highlighted for the treatment of burns and chronic wounds. These naturally occurring or chemically cross-linked three-dimensional (3-D) networks of polymer chains or macromolecules are filled with a significant amount of liquid and provide a supportive environment for tissue regeneration. These materials follow the contours of the wound surface and ensure oxygen and water permeation while protecting the surface from bacterial invasion (Quinn et al., 1985). One naturally derived hydrogel material that is widely used for dressing production is bacterial cellulose (BC).

BC is a polymer produced by some bacteria belonging to the genera *Acetobacter*, *Rhizobium*, *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Salmonella*, *Escherichia*, and *Sarcina* (Shoda and Sugano, 2005). It was originally served as food (nata de coco) in Asia, in form of sweet candies or custards, but its unique properties have also led to its use as a wound dressing. BC production for the specific purpose of wound dressing dates back to the early 1980s (Farah, 1990; Ring et al., 1986). Its use as a wound healing material is governed by its peculiar features: it has a high tensile strength, flexibility, and water holding capacity, a pronounced permeability to gases and liquids, and a great compatibility with living tissues (Czaja et al., 2006a). BC in its pure form also can undergo modifications that can give it tailor-made properties to fulfill all the requirements essential to function as a wound dressing material (Fig. 1). Its high porosity and surface area allows the potential for introduction and release of antimicrobial agents, medicines, and other biofunctional materials (Shah et al., 2013). The presence of chemically reactive sites within its structure provides the additional possibility for the introduction of specific non-native functionalities (Siró and Plackett, 2010). The inclusion of other compounds that can accelerate sore healing, the preparation of BC-based composites, and the chemical reactivity of the polymer chain could all

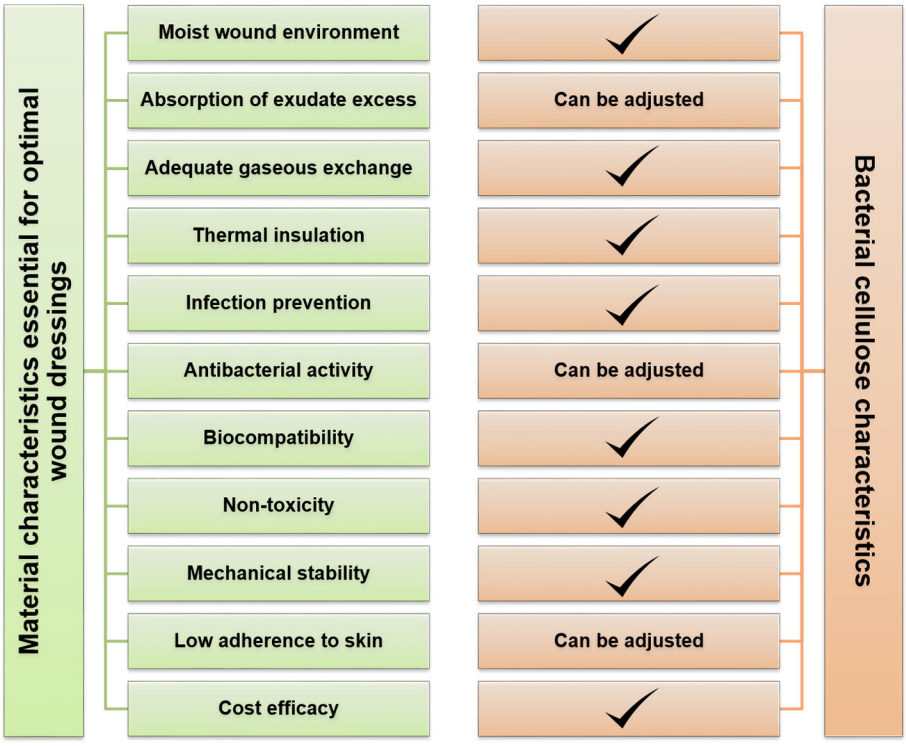

Fig. 1. An overview of bacterial cellulose characteristics with respect to the general requirements for wound dressing materials.

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contribute to the formation of novel dressing materials with improved characteristics for treatment of any type of wound (Hu et al., 2014; Klemm et al., 2011). When used in form of aerogel, BC can be utilized for preparation tissue-engineered products for regeneration of damaged organs as well as for wound care applications. Alike wet BC, aerogels support loading of the active substances and may be used as controlled-release matrices (Haimer et al., 2010).

The present work reviews the various approaches taken to modify BC to enhance its applications in sore restoration by improvements in biocompatibility, water holding capacity, and antimicrobial activity. The substantial problem of adhesion of dressings to the wound and the resulting pronounced discomfort of dressing changes is explored and possible ways to overcome this particular drawback are discussed. Post production modifications of harvested BC pellicles are the main focus of the present review, with brief remarks on the pathways directly exploited for alteration of BC properties during its biosynthesis.

2. Bacterial cellulose — general properties and ways to improve material characteristics

BC, a homopolymer produced by some bacterial strains, has the same chemical structure of linear β-1,4-glucan chains as plant-derived cellulose (Bodin et al., 2011). However, a number of distinctions are found in the chemical and physical properties of the bacterial versus plant polymers. Whereas plant-derived cellulose chains are closely associated with hemicelluloses, lignin, and pectin, BC is free of other polymers (Klemm et al., 2001). During its biosynthesis by bacteria, cellulose chains are polymerized by cellulose synthases A (CesA) from activated glucose. The single chains are then extruded through the bacterial cell wall by rosette terminal complexes. About 50 to 80 extrusion sites are located linearly along the cell long axis and release glucan chains (Ross et al., 1991). The macromolecules assemble into hierarchically organized units as a complex, primarily forming subfibrils of 10–15 glucan chains that assemble to form microfibrils, and finally microfibril bundles (Bodin et al., 2011). The loosely assembled bundles then form cellulose ribbons comprised of about 1000 polyglucan chains. Continuous spinning of cellulose ribbons by bacteria leads to the formation of a highly pure 3-D structure of nanofibers stabilized by inter- and intra-fibrillar hydrogen bonds, as presented in Fig. 2 (Chawla et al., 2009). This structural singularity of the BC fibrillated network results in unique mechanical characteristics, such as a high degree of crystallinity (60–80%) (Czaja et al., 2006a) and a Young's modulus of 15–30 GPa, the highest of all two-dimensional organic materials (Shoda and Sugano, 2005). A high surface area, as result of the high aspect ratio of the fibers, provides a great liquid loading capacity of up to 99 wt.%. In the case of water, about 90% of the water molecules are tightly bound to the large number of hydroxyl groups within the cellulose molecules (Gelin et al., 2007). BC fibers have a greater specific area in comparison to plant derived cellulose fibers. Water absorbency of BC was more than 30% greater than for cotton gauze, and the drying time was 33% longer (Meftahi et al., 2009).

These features of BC impart the properties that are essential for wound healing materials: mechanical stability of the dressings, proper liquid capacity, and material compatibility with living tissues. However, the normal progression of the wound healing mechanism can be disrupted by numerous factors, giving rise to prolonged healing times (Pitzer and Patel, 2011). In such cases requirements to dressing materials rise to a new level demanding its active participation in the complex process of wound recovery (Fonder et al., 2007). The main complications occur in case of chronic wounds that may undergo severe physiological changes or produce tumors. This results in excessive production of exudates containing high levels of tissue-destroying proteinases and in wound contamination by foreign bodies, such as bacterial species, that induce inflammatory responses (Boateng et al., 2008). For example, more than 50% of diabetic chronic ulcers become infected. These infections are one of the most common and severe sequelae in the wound recovery process and are the major factor delaying healing (Alavi et al., 2014). The problem of wound colonization with microorganisms is particularly critical in burn patients due to their adversely affected immune systems, coupled with the extensive disruption of the physical skin barrier that no longer prevents bacterial invasion (Calum et al., 2009).

BC modifications directed to include antimicrobial characteristics may significantly improve the material efficiency in respect to treatment of particularly severe cases, such as chronic wounds, diabetic ulcers and burns (Day, 2011; Eberlein et al., 2012). Improved interaction with living cells may support migration of epithelial cells and fibroblasts and therefore accelerate replacement of the lost tissues (Morgado et al., 2015). Apart from that, dressings with high capacity for exudate adsorption are favorable in chronic wound treatment. Proper moisture control is generally increasing healing rates, protects the wound from infection, reduces pain, and decreases overall health care costs (Agarwal et al., 2011; Day, 2011). Additionally, water absorption and holding capacities provide the possibility of loading liquid drugs and bioactive compounds into the structure of the dressing material (Shah et al., 2013). The ability to retain a moist environment also prevents the dressing from drying and sticking to the wound, thereby protecting the wound from disclosure and reducing pain during dressing exchange (Ovington, 2007). Therefore, BC modifications aiming at improved wound dressing characteristics are mainly related to the enhancement of biocompatibility properties and liquid holding and release capacity together with impregnation with antimicrobial components. Modification approaches related either to the adjustment of BC structural characteristics during biosynthesis process or to post-synthetic material modification through chemical derivatization or preparation of composites (Hu et al., 2014).

The control of biofabrication conditions allows the adjustment of BC fiber composition and morphology as well as the shape, degree of crystallinity, and pore size of the final product (Struszczyk et al., 1995). Diverse parameters affect the network formation: environmental factors such as temperature, pH, dissolved oxygen, and stirring speed of the growth medium as well as cultivation time and cultural media conditions, including the carbon and nitrogen sources and nutrients for microorganism growth and the presence of various additives. Several excellent publications have provided comprehensive overviews on the influence of the agitation conditions on the properties of BC (Chawla et al., 2009; Dufresne, 2012; Keshk, 2014b; Krystynowicz et al., 2002). The cultivation conditions additionally affect the microscale morphology. Under static conditions, bacteria accumulate at the oxygen-rich surface of the nutrient broth to form overlapping, intertwisted ribbons with a low organizational pattern. This morphology can be improved by producing BC in an agitated culture, where the bacteria are well dispersed in the culture medium slurry: BC will be formed as irregular granules and stellate and fibrous strands that have a tendency to form highly branched, reticulated structures (Bielecki et al., 2005b; Esa et al., 2014; Keshk, 2014b). The control of structural characteristics of the material extends the scope of BC utilization in the biomedical field, and may help to improve the properties of BC-based wound dressings, mainly water absorption and water release rates (Ul-Islam et al., 2012b).

Another possibility to alter BC structure and characteristics relates to chemical modification with a view to obtain BC derivatives with novel properties (Hu et al., 2014). The chemical derivatization of plant-derived cellulose has become one of the major areas in cellulose science. The progress in this field has afforded various chemically modified cellulosic products with unique functional properties (Atalla and Isogai, 2010). The main active sites for derivatization reactions are the three hydroxyl groups of the glucose monomer units. Crosslinking and copolymerization reactions, for instance, might lead to the formation of superabsorbent hydrogels and materials with improved mechanical characteristics (Heinze and Liebert, 2012; Pérez and Samain, 2010) that resemble BC gels. Generally, the structural and molecular similarity of cellulose fibers synthesized by bacteria with those extracted from

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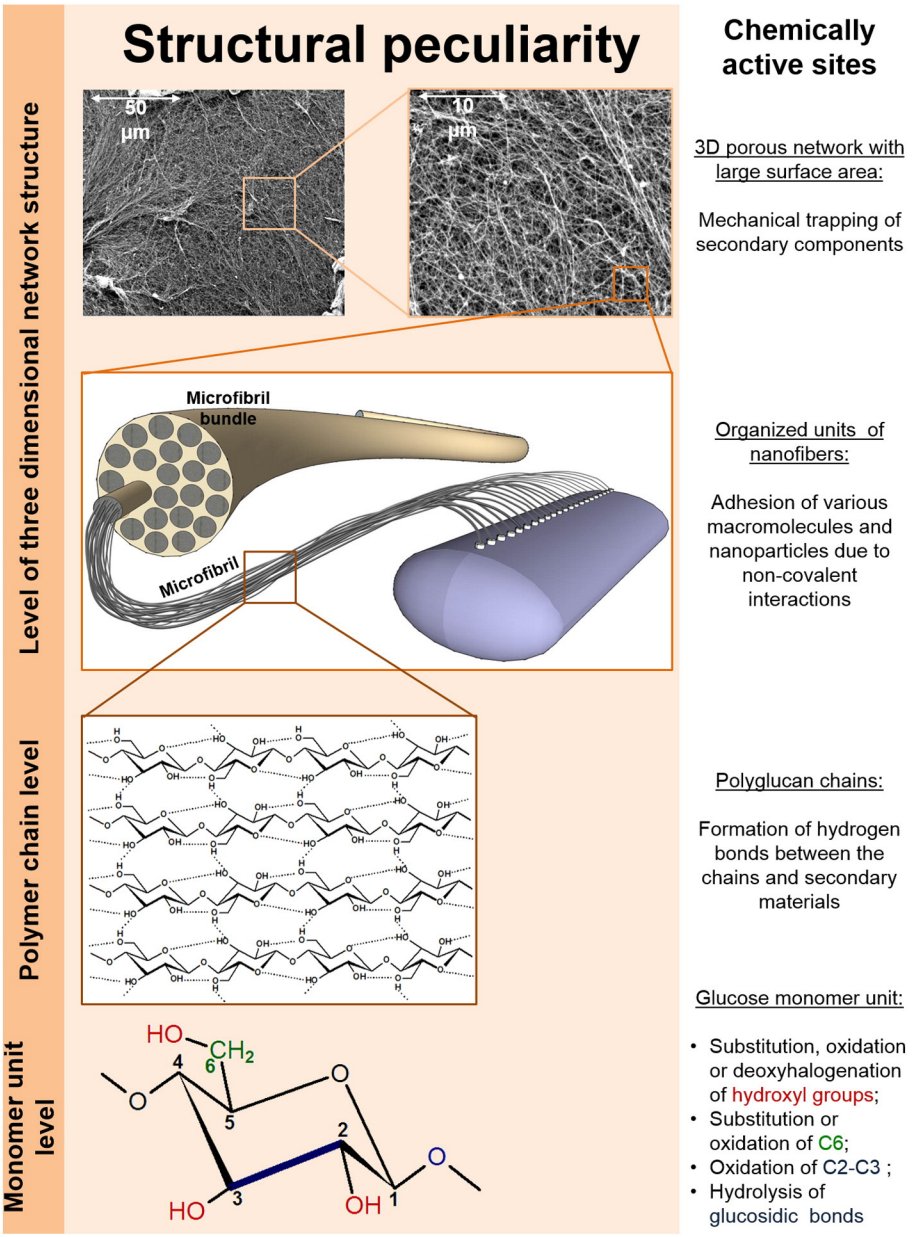

Fig. 2. General overview of bacterial cellulose (BC) structural organization.

plants means that chemical modifications established for plant celluloses should be transferrable to the BC case without problems. Chemical derivatization reactions are mainly applied to BC wound dressing material with a view to introduce covalently bound functional groups which may impart hitherto deficient properties, such as antimicrobial activity (Fernandes et al., 2013). Additionally specific functional groups may lead to better affinity towards secondary components, including bioactive macromolecules or cell lines, improving BC biocompatibility. A larger adsorption of metal ions (Oshima et al., 2008) and proteins (Oshima et al., 2011) onto phosphorylated BC and a greater adsorption of hemoglobin (Niide et al., 2010) onto quaternary ammonium BC was reported in comparison to similarly modified plant-derived cellulose. Sulfate group introduction of up to 0.42% of OSO_3^- groups improved BC interaction with xyloglucan (Pirich et al., 2015). However, all such chemical modifications of BC have a severe drawback: while biocompatibility as well as non-inflammatory and non-toxic properties have been established and widely affirmed for BC (Bodin et al., 2011), such proofs are missing for the new derivatives, and their biomedical characteristics will have to be tested and confirmed in lengthy and costly procedures.

Composites generation is another approach to impart new functionalities to the existing BC-based materials, mainly used to open up new potential applications in diverse areas such as development of electrical products and optoelectronic devices (Jeon et al., 2010; Kim and Seo, 2002). With regard to wound treatment applications, the approach of composite formation is hampered by the same impediment as chemical modification: the unclear general effects of the secondary components and the final composite on biocompatibility and toxicity (Shah et al., 2013). Composite formation mostly occurs through penetration of liquid reinforcement materials or nanoparticles into the BC structural matrix. Mechanical trapping and physico-chemical interactions of these substances with the BC interfibrillar network are the main mechanisms of composites formation (Hu et al., 2014). The microfibrillar nature and the large surface area of BC promote the adhesion of other components of the composite matrix, and the ability to form intermolecular hydrogen bonds provides additional stability to the BC composites. BC was shown to possess a better absorption capacity than plant derived cellulose, as confirmed by a higher adsorption of xyloglucan and cellobiose dehydrogenase (Ougiya et al., 1998). Inclusion of biocompatible and/

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or bioactive compounds as components of the composite is an elegant way to overcome certain limitations of "pure" BC and to come closer to an "even more ideal" dressing material with improved biocompatibility, antimicrobial activity and water holding capacity (Ciechańska, 2004; Kim et al., 2010a; Lin et al., 2013a).

The peculiarities of BC structural organization at different levels, as well as the chemically active sites mainly affected during modifications, are presented in Fig. 2. An overview of possible modification pathways affecting all levels of BC structural organization is presented in Table 1. The table represents a comprehensive selection of general modification approaches during the biosynthesis procedure and in post-treatment of fabricated hydrogels. As the number of available publications is rather large, only selected representative examples are given for each category, and no claim regarding completeness is made.

3. Bacterial cellulose wound dressing — production technology and efficiency in wound treatment

The demand for sterile wound dressing fabrication tolerates several methods of material processing steps. Purification of the harvested pellicles from the residues of the biosynthetic media is the necessary step of the wound dressing production (Fontana et al., 1990). Further modifications are limited regarding the choice of implemented techniques which are imposed by the necessity to preserve the biocompatibility of the final product. The use of toxic chemicals is evidently restricted, and no chemicals except the desired bioactive components should remain in the structure after the modification procedure (Serafica et al., 2010). Nowadays, BC processing steps for wound dressing production are well established and do not impair any material characteristics important for wound recovery support (Bielecki et al., 2005a).

As the initial step, industrial scale production of BC wound dressings requires an efficient purification of raw material to remove the residues of the cultural media and bacterial cells, which may cause wound contamination and induce immune responses (Fontana et al., 1990). The most widely used purification procedure is treatment of the harvested pellicles with alkali (aqueous NaOH; 0.1–1 M) at 60–100 °C for 1–3 h, followed by neutralization with organic acids (e.g., acetic acid) or washing with water until a neutral pH is restored. Peroxide at concentrations of about 0.05–10 wt.% is used in some cases to whiten the films (Bielecki et al., 2005a; Serafica et al., 2010). Other bleaching reagents, such as hypochlorite, hypobromite, or perborate, are also applicable as whiteners (Hoon and Mormino, 2005). Purification procedure is the way to obtain "ideal" cellulose with no impurities from cultivation present in the structure. Aside from immune response, these protein impurities have been shown to additionally influence on the aging behavior of BC causing yellowing of the material (Rosenau et al., 2014). Further modifications, when implemented, are aimed at adjusting the dressing material properties to a specifically desired form. Primarily, this involves impregnation with antimicrobial agents, such as polyhexamethylene biguanide (PHMB) (Serafica et al., 2010), silver nanoparticles (Wan and Guhados, 2009), or photoactive TiO_2 nanoparticles (Limaye et al., 2009). A wide variety of antimicrobial BC-based dressings are currently available in the market, but the choice of bicomponent systems containing a second bioactive component together with BC is rather slim. Sterilization of the BC based product after all the processing steps is a final phase of wet dressing preparation. Autoclaving, gamma irradiation, or electron beam sterilization may be used to obtain a final product suitable for wound or burn treatments (Ring et al., 1987; Serafica et al., 2010). Although sterilization is known to affect BC structure causing some chain scission and thus a slight decrease of the molar mass values, it is an inevitable step of medical wound dressing production (Jipa et al., 2012; Wanichapichart et al., 2012).

The efficiency of BC based wound dressing as a material supporting tissue regeneration has been investigated in extensive medical studies (Kwak et al., 2015; Muangman et al., 2011). Rapid and efficient healing of severe chronic and burn wounds has been repeatedly reported by different research groups. Some profound review articles provide an overview on these studies (Czaja et al., 2006a; Fu et al., 2013). Comparative studies have demonstrated the advantages of BC dressings over petrolatum gauze in the treatment of troublesome diabetic foot ulcers. Utilization of BC shortened the mean wound healing time and the rate of weekly wound closure (Solway et al., 2011). Additionally, the advantages of BC dressings over standard wound care systems (silver sulfadiazine cream) could be shown in treatments of burns (Piatkowski et al., 2011) and skin tears of the frail elderly (Solway et al., 2010). The economic benefit of using BC dressings instead of conventional synthetic fiber dressings (e.g., surgical pads, tulle grass, and saline-soaked gauze) and other moisture-ensuring wound dressings (e.g., synthetic foams and alginate dressings) has been confirmed by long-term clinical studies (Schmitz et al., 2014). The effectiveness of BC is primarily associated with the fact that advanced wound healing products should require only a low number of dressing changes over the healing period, resulting in cost reduction (apart from the evident benefit for the patient). Cost savings from the use of BC in combination with secondary dressings over three months was between 60–70% when compared to traditional dressings (Schmitz et al., 2014).

4. Biocompatibility of bacterial cellulose

The major requirement for consideration when developing any new biomedical material is its compatibility with living tissue materials. The conventional meaning of biocompatibility includes an appropriate host response to the new material in every specific situation, as well as the absence of toxic effects or tissue injury (Williams, 1999). The compatibility of BC is associated with its peculiar nanofibrillar structure: its 3-D porous network supports cell proliferation and penetration (Czaja et al., 2006a). The biocompatibility of different types of BC-based implants with living tissues has been observed on repeated occasions (Table 2). The absence of a foreign body reaction and the low inflammatory reaction at the first stage after implantation indicate good biocompatibility characteristics of the BC as a material for medical applications. Implants demonstrate suitable integration with the host tissue and support the adhesion and infiltration of the host connective tissue cells (Klemm et al., 2001). The biocompatibility of BC is comparable to that of other biomaterials widely used for tissue engineering, such as polyglycolic acid and polytetrafluorethylene. Implantation of dorsal skinfold chambers in Syrian golden hamsters demonstrated that BC was comparable to the control samples in terms of biocompatibility, cell proliferation support, and suppression of apoptotic cell death (Esguerra et al., 2010).

BC implanted into living organisms supports adhesion and ingrowth of the connective tissue cells, indicating the good biocompatibility of the material. Multiple cell seeding experiments have explored the interactions of native BC. BC is a good cell-supporting matrix that maintains suitable conditions for good proliferation of different kind of cells. An overview of the diversity of tested cell cultures is shown in Table 3.

The ability of specific cell colonies to infiltrate BC suggests that adaptive remodeling of BC grafts *in vivo* could achieve better compliance in the long term by matching the BC with the host tissues. The BC implants were also shown to possess proper blood biocompatibility without clotting acceleration and hemolysis (Zang et al., 2015). Taken together this would widens the scope of the potential applications of BC-based scaffolds: BC has been claimed to be suitable for some specific uses, such as tissue engineering of the urinary conduit (Bodin et al., 2010). The possibility of inducing pluripotent stem cells indicates great potential for BC use in the field of regenerative medicine. The recent discovery of iPSCs has introduced a powerful method for cell differentiation and may fulfill unmet needs in the field of tissue engineering (de Oliveira, 2012).

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Table 1
Synoptic table presenting an overview of the possible pathways for modification of bacterial cellulose (BC) properties.

***In situ* modifications during the biosynthesis process**			***Ex situ* modifications of bacterial cellulose hydrogel**		
	Affected site	**Obtained effect**	**Affected site**	**Obtained effect**	
Monomer unit level Monomer chain formation	• Control of chain length		• D-glucose monomer unit		**Monomer unit level** **Covalent interactions** of the reactive sites
	1. Variation of fermentation time	Non-linear dependence of DP from harvesting time was observed by Shi et al. (2012a)	1. Substitution reaction		
	2. Changing biofabrication conditions	Fermentation in trickling reactor results in higher DP (Lu and Jiang, 2014)	*Esterification* with alkyl ketene dimer	Hydrophobic polymer-brush type BC β-ketoesters (Yoshida et al., 2012)	
	3. Use of different carbon sources	Glucose as a carbon source results in a higher DP in comparison with glycerin and xylose (Shi et al., 2012a)	impregnation of Fmoc-Glu	Improved interaction of BC with amino-containing materials (e.g. collagen) (Saska et al., 2012)	
	4. Addition of components to the agitated culture	Waste glycerol medium supplemented with carboxymethylcellulose leads to the formation of nanofibrillated cellulose (Kose et al., 2013)	*Etherification* with alkyl bromides	Ethyl-, propyl-, i-propyl-, n-butyl BC derivatives with better solubility in low polar solvents (Lin et al., 2013b)	
			carboxymethylation	Improved water solubility (Geyer et al., 1994); Improved protein adsorption (Lin et al., 2015a)	
			hydroxypropylation	Hydrophobization of BC surface (Chen et al., 2014a)	
			epoxy group BC ether	Active site for further modifications (Lu et al., 2013)	
			Acetylation	Carbon reactivity: C(6)>>C(2)>C(3) (Lima et al., 2011); Control of DS by changing reaction time (Barud et al., 2008)	
			Phosphorylation	BC with improved adhesion of proteins (Oshima et al., 2007), hydroxyapatites (Wan et al., 2007), and metal ions (Oshima et al., 2008)	
			Sulfation	Different DS, significant degradation (Zhu et al., 2014b)	
			Silylation hexamethyldisilazane	Improved solubility in THF, benzene and $CHCl_3$ (Geyer et al., 1994)	
			aminopropyltrimetoxysilane	Biocompatible material with antimicrobial activity (Fernandes et al., 2013)	
			Nitration	Different DS, carbon reactivity: C(6)>C(2)>C(3) (Yamamoto et al., 2006)	
			Iodination	Triester hypoiodous BC (Keshk, 2008)	
			Succinylation	Material for metal ions adsorption (Cu^{2+}) (Yin et al., 2010)	
			2. C(6) oxidation to carboxyl (TEMPO or NO_2/HNO_3)	Improved reactivity towards amino-containing polymers (chitosan) (Lai et al., 2014; Xu et al., 2014), alginate (Park et al., 2015a), proteins (Cui et al., 2014) and Ag nanoparticles (Ifuku et al., 2009); Reduced interfibrillar adhesion, increased surface free energy (Lai et al., 2013)	
			3. C(2)-C(3) oxidative cleavage	Material with improved cell adhesion and proliferation degrees (Wu et al., 2014b); Improved reactivity with antibiotics (Lacin, 2014)	
			• β-(1,4)-glucosidic bonds		
			1. Acid hydrolysis	Microcrystalline cellulose with DP of 250 (de Oliveira et al., 2011); Formation of nanofibrils and nanocrystals (Sacui et al., 2014); BC nanowhiskers (Wang et al , 2014a)	
			2. Enzymatic hydrolysis	Cellulose nanocrystals (George et al., 2011)	
Polymer chain level Microfibril formation and interaction with media components	• Control of fiber diameter		• Impregnation with secondary components		**Polymer chain level** **Non-covalent interactions** of BC microfibrils with other substances
	1. Changing fermentation time	Non-linear dependency of microfibril diameter from duration of cultivation (Luo et al., 2014b)	1. Inorganic substances		
	2. Changing biofabrication conditions	Shaking conditions result in thinner and longer BC fibers (Mohite and Patil, 2014)	*Hydroxyapatite (HyAp)*	Biomaterial imitating bone tissue (Wan et al., 2007)	
	3. Addition of other components to the agitated culture		*HyAp/Graphene oxide*	Biocompatible material with osteoinductive potential (Ramani and Sastry, 2014)	
			Calcium phosphate	Materials with dielectric properties (Khayrullin et al., 2013)	
	Carboxymethylcellulose (CMC)	50% loss in fiber diameter (Grande et al., 2009a)	2. Metals, including salts and oxides		
	Single α-sugar-linked glucuronic acid-based oligosaccharide	Increased microfibril diameter (Ha et al., 2011)	*Ag*	Antimicrobial BC based composite (Maneerung et al., 2008)	
			Pd	Thermally stable membranes with catalytic activity (Evans et al., 2003)	
	• Interactions with media additives		*Au*	Material suitable as a substrate for SERS (Park et al., 2013); Preparation of biosensors for detection H_2O_2 (Zhang et al., 2010)	
	1. Inorganic substances		*Cu*	Suitable material for development electronic paper technologies (Pinto et al., 2012)	
	Graphene	Composite with suitable electrical properties (Luo et al., 2014a)	*Pd/Cu*	Catalyst for water denitrification (Sun et al., 2010)	
	Hydroxyapatite	Scaffold with improved biocompatibility for bone regeneration (Grande et al., 2009a)	Fe_3O_4	Composite with superparamagnetic behavior and electrical permittivity (Marins et al., 2013)	
	Fe_3O_4	Material adsorbent for heavy metals (Zhu et al., 2011)	*ZnO*	Antimicrobial bionanocomposite foams (Wang et al., 2014b)	
			ITO (Indium Tin Oxide)	Substrate for OLED displays fabrication (Legnani et al., 2008)	
	2. Biopolymers		La_2CuO_4	Catalyst for methanol steam reforming reaction(Yang et al., 2013)	
	Collagen	Material with high efficiency in wound healing (Wiegand et al., 2006)	*ZnS*	Composite with photocatalytic activity (Zheng et al., 2014)	
			LiCr	BC with higher ionic conductivity(Jeon et al., 2010)	
	Chitosan	Promising material for wound healing therapy (Fu et al., 2014; Phisalaphong and Jatupaiboon, 2008)	3. Biopolymers		
			Collagen	Improved thermal stability, Young's Modulus and tensile strength (Zhijiang and Guang, 2011)	
	Chitin, partially deacetylated	Composites with great antimicrobial activity (Butchosa et al., 2013)	*Chitosan*	Transparent, flexible, biodegradable films (Fernandes et al., 2009); Biocompatible and antimicrobial active composite (Kingkaew et al., 2014)	
	Starch	BC with improved mechanical characteristics (Grande et al., 2009b)	*Alginate*	Biocompatible material efficient in wound healing (Chiaoprakobkij et al., 2011); Sponges for yeast cell carrier for EtOH production (Kirdponpattara and Phisalaphong, 2013)	
	Aloe vera gel	Films with improved mechanical characteristics, water absorption and transmission features (Saibuatong and Phisalaphong, 2010)			
			4. Synthetic polymers		
	Alginate	Films with improved water capacity and reduced O_2 permeability (Kanjanamosit et al., 2010)	*PVA*	Anisotropic composites with high tensile strength (Millon et al., 2008)	
	Hyaluronic acid	Possibility to obtained material with tailored hydrophobic/hydrophilic properties (Lopes et)	*Polyurethane resin*	Membrane for OLED displays fabrication (Ummartyotin et al., 2012)	

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Table 1 (*continued*)

	3. Synthetic polymers *Poly(vinyl alcohol) (PVA)* *Poly(ethylene oxide) (PEO)*	 Transparent films with improved mechanical and optical properties (Gea et al., 2010); Composite with improved water retention ability and ion absorption (Seifert et al., 2004) Fiber-reinforced thermoplastic nanocomposites (Brown and Laborie, 2007)	*Polypyrolle* *Poly(3,4-ethylenedioxy thiophene)/ poly(styrenesulfonate)* 5. Antimicrobial components *Silver sulfadiazine* *Octenidine* 6. Proteins for peptide adhesion *RGD-carrying peptide* 7. Dye affinity ligands *Reactive Green 5* 8. Combinations of reagents *Rhodamine 6G / Si or Ag NPs*	Membranes with high electrical conductivity (Xu et al., 2013) Electro-active hybrid actuators consisting of BC between conducting polymeric layers (Kim et al., 2013) Materials with advanced antimicrobial activity for wound therapy (Luan et al., 2012; Moritz et al., 2014) Materials for biomedical applications with improved biocompatibility towards desired cell lines (Andrade et al., 2010a) Material with selective absorbance efficiency towards proteins (e.g. urease) (Akduman et al., 2013) Material with random laser emission (dos Santos et al., 2014)	
3-D network level Control of microfibril network organization	• Control of crystallinity 1. Changing of biofabrication conditions 2. Use of different carbon sources 3. Addition of other components to the agitated culture *Carboxymethylcellulose* *Hydroxypropylmethylcellulose* *Alginate* *Xyloglucan* *Poly-3-hydroxybutyrate* *Tween 80* *Vitamin C* • Control of porosity 1.Changing of fermentation time 2. Changing of biofabrication conditions 3. Use of different carbon sources 4. Addition of other components to the agitated culture *Carboxymethylcellulose* • Control of 3-D morphology 1. Under electric field 2. Local prevention of biosynthesis with electric pulses 3.Cultivation on PDMS substrate 4. Cultivation on O_2-permeable silicone substrate 5. Cultivation on honeycomb-patterned agarose scaffold	 Shaking conditions lead to higher crystallinity composed by high Iα structure (Mohite and Patil, 2014); Crystallinity from fed-batch cultivation was shown to be higher than those from static culture (Shezad et al. , 2010) Crystallinity degree is decreasing when using sucrose << glucose ≈ maltose < fructose (Yang et al., 2012; Zeng et al., 2011) Reducing of crystallinity due to the interaction of BC fibrils with the additives and decreased interfibrillar hydrogen bonding formation (Cheng et al., 2009) (Ruka et al. , 2013) (Cheng et al., 2009) Additionally decreasing Iα/Iβ allomorph ratio (Park et al., 2014) (Ruka et al., 2013) (Ruka et al., 2013) (Keshk, 2014a) Non-linear increasing of porosity within 2 week of fermentation (Luo et al., 2014b) Trickling bed reactor results in superior porosity (Lu and Jiang, 2014); Smaller pore size detected after fabrication in static culture in comparison with agitated (Guo and Catchmark, 2012) Pore size decreasing when using sucrose << glucose ≈ maltose ≈ fructose ≈ glycerol (Tang et al., 2009) Reducing of porosity (Ma et al., 2014) Formation of network with aligned fibers (Sano et al., 2010) Local pore formation for the preparation of the scaffolds (Baah-Dwomoh et al., 2015) BC gel with anti-axially oriented fibrils (Putra et al., 2007) Uniaxial fibril orientation along the longitudinal axis of silicone tube (Putra et al., 2008) Highly oriented BC microfibrils along substrate pore walls (Uraki et al., 2007, Zang et al., 2014)	• Control of porosity 1. Influence of post fabrication treatment 2. Plasma treatment • Adjustment of 3-D morphology 1. Laser formation of micropattern 2. Use of foaming agents 3. Micropore generation with porogens *Potato starch* *Agarose* *Paraffin wax microspheres* • Control of microfibril orientation 1. Direct melt spinning of BC nanocrystals dissolved in low molecular weight sugar alcohol blend (Isomalt®)	Pore size id decreasing after alkali post treatment in comparison to thermal treatment (Santos et al., 2015) Porosity decreased when treated with K_2CO_3 > Na_2CO_3 > KOH > NaOH alkali solutions (Tang et al., 2009) Treatment with nitrogen-containing plasma increasing the porosity (Pertile et al., 2010) Generation of round-shaped microchannels via pulsed CO_2 laser treatment (Ahrem et al., 2014) Formation of orderly open-microporous structure (Yin et al., 2012) Removal of components presented in fermentation media from the harvested pellicles Improved porosity with up to 40 μm pores (Yang et al., 2014) Uniformly distributed 300-500μm pores (Yin et al., 2015) 300–500 μm interconnected pore structure (Zaborowska et al., 2010) Obtained fibers after removal of Isomalt matrix possess high oriented microstructure (Bizot and Cathala, 2014)	**3-D network level** Formation of microporous structures and fiber orientation control

Besides its good biocompatibility, BC also shows low cytotoxicity or genotoxicity. The potential toxicity of BC has been studied using BC nanofibers obtained by chemical and/or mechanical treatment of BC membranes in *in vivo* and *in vitro* experimental setups. Subcutaneous implantation of these BC nanofibers in BALB/c mice did not change the normal development of the animals; no differences were noted in histological analyses of the internal organs between implanted and control animals (Pertile et al., 2011). No toxicity in the liver and kidney organs of SD rats was observed when the rat model was used to study the efficiency of BC wound dressings (Kwak et al., 2015). The absence of cytotoxic effects of BC nanofibers on either cell apoptosis or the cell cycle has been confirmed in *in vivo* studies by Jeong et al. (2010) using C57/Bl6 mice and in *in vitro* studies on human umbilical vein endothelial cells (HUVECs). Another test with HUVECs demonstrated similar results, as single cell gel electrophoresis and *Salmonella* reversion assays showed no toxicity of BC nanofibers under the condition tested (Moreira et al., 2009). Bodin et al. (2010) incubated porous BC scaffolds in water with the view of determining endotoxin levels. The detected values were low, at ≤0.1 ± 0.04 endotoxin units. The limit for endotoxins for medical devices established by the Food and Drug Administration (FDA) is 0.5 EU/ml (based on a 40 mL rinse). Martínez Ávila et al. (2014) also detected low endotoxin levels (below 0.1 EU/ml) after a depyrogenation process including the rinsing of BC samples with endotoxin-free water for 14 days.

Application of BC in wound management requires that there be no skin reaction to the material. The skin tolerance of BC materials and the outcomes of using BC for wound therapy have been investigated. A good *in vivo* skin compatibility of BC membranes produced by *Gluconacetobacter sacchari* was determined in human subjects, whose erythema clinical score were zero at 2 and 24 h after patch removal in almost all the volunteers (Almeida et al., 2014). Studies on skin lesion treatment of BALB/c mice confirmed the advantages of using BC films, including fast rehabilitation of the wound surface together with reduced inflammation, ease of dressing removal, suitable mechanical properties, and high biocompatibility that ensured good protection of the wound and a proper environment for cell proliferation (Fu et al., 2012). Similar

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Table 2
In vivo studies on the biocompatibility of bacterial cellulose (BC)-based implants.

BC sample type	Experimental model	Experiment duration	Inflammation reaction	Foreign body reaction	Comments	Refs
BC pellicles purified with NaOH	Hypodermic implantation in Wistar rats	12 weeks	No	No	Infiltration of the fibroblasts from the host tissue; Formation of the new blood vessels in and around the implant	Helenius et al., 2006
BC tubes, Ø 1.0–3.0 mm	Vascular implants in carotid arteries of white rats	12 months	–	–	Endothelial cells preserved their entire function in the neointima; Ingrowth of active fibroblasts	Schumann et al., 2008
BC tubes, Ø 3.0–3.7 mm	Implanted into the carotid arteries of white domestic pigs	3 months	–	–	Integration with endothelium, collagen, smooth muscle cells and fibrous cells; Good compatibility with the tissue of the high blood pressure animals	Schumann et al., 2008
BC membranes sterilized by γ-radiation	Subcutaneous implantation into lumbar tissue of Swiss Albino mice	90 days	Mild inflammatory response within first 30 days	No	No connective tissue cells surrounded the samples	Mendes et al., 2009
BC membranes, purified with SDS and NaOH	Subcutaneous implantation into the backs of BALB/c mice	12 months	Mild inflammatory response decreasing overtime	No	Calcification effect correlated with the porosity of BC samples	Pertile et al., 2011
Kampuchea-syn-thesized BC tubes, Ø1 mm; 10 mm length	Nerve conduit implanted into the spatium intermusculare of SD rats	6 weeks	Mild inflammatory response decreasing overtime s	No	At six weeks post surgery, the implants showed a clear interface with surroundings with just very few inflammatory cells	Zhu et al., 2014a
BC tubes, 100 mm length, Ø 4.0–5.0 mm	Implantation into carotid artery of Texel sheep	12 weeks	No signs	No	Formation of a vascular wall-like structure along the scaffold and a homogeneous endothelialization of the inner surface of the implant	Scherner et al., 2014
Disc-shaped densified BC pellicles (Ø10 mm)	Auricular cartilage implanted into Chinchilla Bastard rabbits	1 week	Yes	Minimal response	Changes in material density affects the surface morphology and determines the level of the foreign body reaction	Martínez Ávila et al., 2014
BC tubes, Ø 6–4 mm, 1 mm thickness, 100 mm length	Implanted in the New Zealand rabbit femoral artery	1 month	No signs	–	Tubes exhibit complete endothelialization with a confluent endothelial layer	Zang et al., 2015
BC pellicles round shaped in size of the wound	Wistar rats with full-thickness skin injures	14 days	Yes	–	The application with the more porous side of the dressing to the wound results in faster skin regeneration	Li et al., 2015

observations of positive effects of the use of BC on epidermis lesions have been repeatedly reported (Czaja et al., 2006b, 2007; Farah, 1990; Fontana et al., 1990).

5. Improved cell interactions

In vivo and *in vitro* studies on BC materials have demonstrated suitable degrees of cell spreading and proliferation. However, these parameters depend on the physical and chemical characteristics of BC, such as its pore size distribution (Zaborowska et al., 2010), fiber morphology (Helenius et al., 2006), and the presence of novel reactive sites introduced through chemical modifications (Pertile et al., 2010). The reasons for the observed changes in cell interactions with a functional extracellular environment lie in the sophisticated nature of this complicated process. This interaction is a highly complex act regulated by the unique physicochemical characteristics of the medium (de Sousa Andrade et al., 2015). The extracellular matrix (ECM) in living tissues provides signals of different natures to the cells, originated by biochemical compounds contained within it (Henke et al., 2012). These physical signals are provided by the insoluble intricate gel-like network of macromolecules that constitute the ECM, including fiber-forming protein chains (e.g., collagen, elastin), glycoproteins (e.g., fibronectin, laminin), and hydrophilic proteoglycans (PGs) carrying glycosaminoglycans (GAGs) as side chains. Aside from matrix-forming polymers, soluble macromolecules present in the ECM (e.g., growth factors, cytokines, and chemokines) provide the signals that induce cell adhesion. Diverse chemical, electrostatic, and hydrophilic interactions result in cell coupling, together with specific interactions with protein receptors (Alberts et al., 2002). However, the influence of the ECM is not limited by these biochemical and physicochemical signals; the surrounding cells and tissues also elicit mechanical stimuli, such as stresses and strains. The physical and morphological properties of the matrix (e.g., stiffness, surface structure) can also affect the extent of cell penetration and proliferation (Vincent and Engler, 2011). Complex combinations of extracellular components interact with intracellular bioprocesses to regulate gene expression and govern the fate of all cell processes, such as proliferation, migration, differentiation and apoptosis (Thasneem and Sharma, 2013). Fig. 3 illustrates the interactions that occur between the components of the intercellular matrix.

The disclosure of the intricate nature of cell–matrix interactions may facilitate the search for possible ways to tailor the biocompatible properties of new biomaterials (Waldeck and Kao, 2011). Different strategies, drawn from nature, have already been effectively implemented with different natural and synthetic biomaterials to elicit desired cellular responses. The impregnation of naturally occurring ECM cell adhesion molecules (proteins, polysaccharides) (Romano et al., 2011; Shekaran and Garcia, 2011) and surface patterning (Kane et al., 1999) are some examples. Numerous methods can be implemented to modify BC to facilitate desired direct cell interactions (Torres et al., 2012). Classification of all possible pathways that could affect BC biocompatibility has revealed four major directions in modification. Cell adhesion can be controlled by: (i) changing BC physical properties, such as porosity, fiber diameter, wettability; (ii) chemical derivatization that changes the material's surface properties (e.g., charge, introduction of functional groups); (iii) impregnation with specific cell adhesion molecules (e.g., proteins, peptides); and (iv) preparation of composites by impregnation with a secondary component that shows higher affinity to cell cultures. Table 4 presents an overview of key directions in BC modification aimed at improving its biocompatibility.

5.1. Alteration of physical properties

The physical properties of a material–mainly its surface morphology, fiber structure, porosity and elasticity–determine its biocompatibility (Vincent and Engler, 2011). Studies on synthetic surfaces have

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Table 3
In vitro cell seeding experiments using bacterial cellulose (BC) scaffolds.

Type of cell culture	Comments	Refs
Human adipose derived stem cells (hASCs)	Good adhesion on the BC film	Fu et al., 2012; de Oliveira, 2012
Human urine derived stem cells (hUSCs)	Great proliferation and infiltration into the scaffold; Cell differentiation into urothelial and smooth muscle cells	Bodin et al., 2010
Equine-derived bone marrow mesenchymal stem cells (EqMSCs)	Proliferation on the BC surface and differentiation into osteogenic and chondrogenic cell lines	Favi et al., 2013
Induced pluripotent stem cells (iPSCs)	Good cell adherence, viability, and proliferation	de Oliveira, 2012
Bovine smooth muscle cells (SMCs)	Good adherence	Zahedmanesh et al., 2011; Zang et al., 2015
Endothelial cells (ECs)	Good adherence	Zahedmanesh et al., 2011
Human keratinocytes (HaCats)	Good attachment and spreading	Chiaoprakobkij et al., 2011
Human umbilical vein endothelial cells (HUVECs)	Vigorous growth with normal morphology and arrangement	Zang et al., 2015
Fibroblasts	Good attachment and spreading	Chiaoprakobkij et al., 2011; Li et al., 2015; Nwe et al., 2010; Wang et al., 2009; Xi et al., 2009; Zang et al., 2015
Chondrocytes	Cell proliferation on native, phosphorylated, and sulfated BC	Wang et al., 2009; Xi et al., 2009; Svensson et al., 2005
Embryonic kidney cells (HEKs)	Cell proliferation on native BC, BC synthesized with 1% CMC and nanocomposites of BC-hydroxyapatite	Grande et al., 2009a
Madin-Darby canine kidney (MDCK) cells	Better cell migration on the porous side of the BC	Li et al., 2015
Mouse leukaemic monocyte macrophage cells (RAW 264.7)	Better cell migration on the porous side of the BC	Li et al., 2015
Peripheral nerve-derived Schwann cells	No significant cytotoxic effects or changes in cell functions	Zhu et al., 2014a
Epidermal cells	Cell spreading and growth	Luan et al., 2012

demonstrated a direct and comprehensive effect of nanogratings, nanoposts, and nanopit arrays on cell orientation and migration and on the production of organized cytoskeletal arrangements (Flemming et al., 1999). Complete cell integration requires a permeable structural matrix, so that lower fiber density and higher porosity facilitates cell penetration. These factors also regulate the diffusion of metabolites and oxygen transportation, thereby positively affecting tissue regeneration (Buxboim and Discher, 2012; Chen et al., 2014b; Roach et al., 2007). Compared to smooth surfaces, the 3-D network structure of BC imparts favorable conditions for cell adhesion. However, the inhomogeneous distribution of the pores with diameters of 20–100 nm is not an advantageous feature for cell ingrowth due to a mismatch with the physical dimensions of the mammalian cells, hence penetration and migration into the BC substrate is limited (Yin et al., 2015). Morphological investigations of BC have demonstrated that material cultivation under static conditions leads to the formation of asymmetric membranes with dense upper sides and gelatinous lower layers (Klemm et al., 2001). Similar observations were made by Backdahl et al. (2006), who investigated the denser surface side of a BC pellicle formed at the medium/air transition zone, and discovered small amounts of completely dense

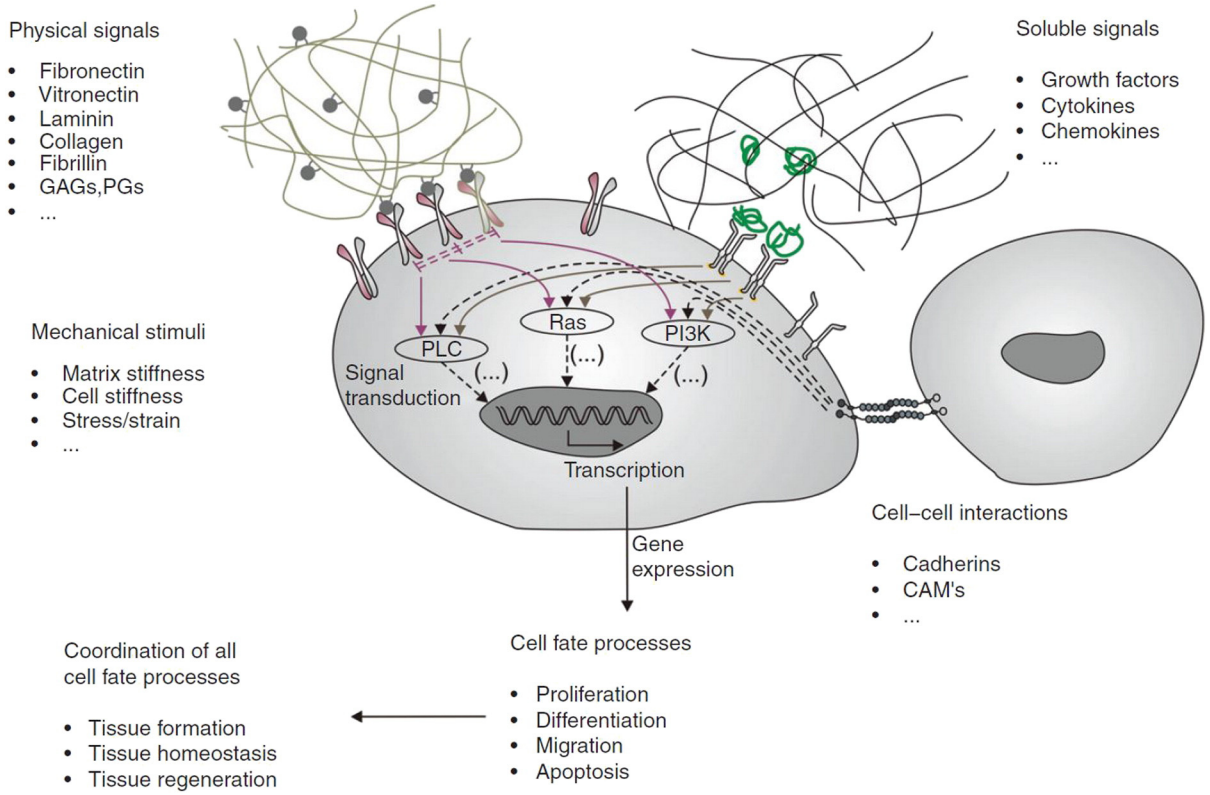

Fig. 3. The complex system of intercellular and extracellular factors affecting cellular behavior. Scheme taken from Henke et al. (2012).

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areas disassociated from more porous regions (Fig. 4). Helenius et al. (2006) observed an influence of inhomogeneous dense distributions on cell penetration. The cells migrated to a deeper degree into the porous side as compared to a compacted side. SEM micrographs revealed cells pushing away nanofibrils to penetrate inside the material. Li et al. (2015) observed better viability and proliferation of the Madin-Darby canine kidney cells, mouse embryonic fibroblast and mouse leukaemic monocyte macrophage cells on the porous side of the BC. Recent *in vivo* studies on wound healing rates confirmed the better performance of BC wound dressings when applied with its porous side in comparison to the more compact side of the dressing (Li et al., 2015).

The limitations attributed to insufficient pore size of BC at the post-processing stage can be overcome by several ways, including pore control during the biosynthesis process by the addition of porogens to the cultivation mixture or by the generation of pore arrays via laser perforation. The purification conditions for harvesting BC membranes can also affect the porosity of the final product. The pore size decreases when the following alkaline solutions were applied: $K_2CO_3 > Na_2CO_3 > KOH > NaOH$. This effect was apparently related to the swelling ability of the reagent, which increased the fibril diameters and thereby reduced the effective pore size (Tang et al., 2009). A recent investigation, however, showed controversial results: Santos et al. (2015) observed an increase of total porosity after alkali treatment of BC with NaOH. This was assigned to the fact that purification helps to release space normally occupied with microorganisms. The contradictory results obtained by Tang et al. (2009) and Santos et al. (2015) may be attributed to the different processing conditions for purification and to different approaches used for pore size distribution measurements.

Porogens of various sizes, such starch (Backdahl et al., 2008; Yang et al., 2014), agarose (Yin et al., 2015) or paraffin wax (Andersson et al., 2010; Backdahl et al., 2008; Zaborowska et al., 2010) microspheres, generate materials with microscale pores of governed size. Addition of a porogens to a growing culture during the BC biosynthesis process can regulate the pore size and porosity of the final structure. Various cell lines, such as human smooth muscle cells (hSMCs) (Backdahl et al., 2008), human chondrocytes (Yin et al., 2015), neonatal articular chondrocytes (Andersson et al., 2010), and MC3T3-E1 osteoprogenitor cells (Zaborowska et al., 2010), have demonstrated better proliferation behavior on microporous BC surfaces. More sophisticated approaches for engineering the architecture of BC involve the use of foaming agents, such as azodicarbonamide, one of the most favored nontoxic foaming agents (Yin et al., 2012), or gamma irradiation

Table 4
Main directions of BC modification aimed at improving material biocompatibility.

Changing material physical characteristics	Derivatization reactions to alter surface properties	Impregnation with special cell adhesion molecules	Impregnation with secondary components with better cell affinity
1. Increasing pore size distribution: • Alkali treatment (Santos et al., 2015) • Addition of porogens during biosynthesis (Andersson et al., 2010; Backdahl et al., 2008; Zaborowska et al., 2010 Yin et al., 2015) • Local prevention of bacteria activity during biosynthesis with electic pulses (Baah-Dwomoh et al., 2015) • Laser perforation (Ahrem et al., 2014; Jing et al., 2011) • Plasma treatment (Pertile et al., 2010) • Gamma irradiation (de Olyveira et al., 2013) 2. Artificial formation of material morphology: • With foaming agents (Yin et al., 2012) • Through emulsion freeze-drying (Gao et al., 2010) 3. Surface topography during the biosynthesis: • With PDMS mold (Bottan et al., 2015)	1. Introduction of cell response stimulating functional groups, (e.g., NH_3-, COOH-, C(O)H-, etc.): • Plasma treatment (Kurniawan et al., 2012; Pertile et al., 2010) • Characteristic chemical reactions: oxidation (Nge et al., 2010) alkylation (Fernandes et al., 2013) acetylation (Gonçalves et al., 2015) 2. Surface hydrophobization: • Plasma treatment (Kurniawan et al., 2012) • Via chemical reactions: grafting of methyl terminated octadecyltrichlorosilane (Taokaew et al., 2015) grafting of amine terminated 3-aminopropyltriethoxysilane (Taokaew et al., 2015)	1. RGD peptides: • Attachment through cellulose binding modules (Andrade et al., 2012) • Attachment through xyloglucan binding modules(Bodin et al., 2007) 2. IKVAV peptide sequence: • Attachment through carbohydrate binding modules (Pertile et al., 2012) 3. ε-Polylysine: • Penetration of a solution into the BC matrix followed by crosslinking with procyanidins (Gao et al., 2011) 4. Poly-L-lysine hydrobromide: • Penetration of a solution into the BC matrix (Culebras et al., 2015) 5. Macrophage-stimulating protein: • Penetration of a solution into the BC matrix (Zhao et al., 2015) 6. Silk fibroin: • Penetration of a solution into the BC matrix (Olivera Barud et al., 2015) 7.Growth factors: • Penetration of a solution into the BC matrix (Lin et al., 2011; Shi et al., 2012b)	1. Collagen: • Penetration of a solution into the BC matrix (Zhijiang and Guang, 2011; Culebras et al., 2015) 2. Gelatin: • Penetration of a solution into the BC matrix (Kim et al., 2010a; Lin et al., 2015b) • In situ generation on BC fibers (Wang et al., 2012b) 3. Chitosan: • Penetration of a solution into the BC matrix (Gonçalves et al., 2015; Kim et al., 2010b; Kingkaew et al., 2014; Lin et al., 2013a) 4. Lecithin: • Penetration of a solution into the BC matrix followed by crosslinking with proanthocyanidin (Zhang et al., 2015) 5. Alginate: • Homogenization of a BC slurry with an alginate solution (Chiaoprakobkij et al., 2011) 6. Polyhydroxyalkanoates: • Insertion of BC microcrystals into a polymer solution (Basnett et al., 2012) • Penetration of a solution into the BC matrix (Zhijiang et al., 2011, 2012) 7. Synthetic polymers, e.g. PEG, PHEMA • Penetration of a solution into the BC matrix (Cai and Kim, 2009; Kim et al., 2010a) • *In situ* radical polymerization (Figueiredo et al., 2013) 8. Carbon nanotubes: • Penetration of a solution into the BC matrix (Park et al., 2015b) • *In situ* impregnation into BC structure during the biosynthesis (Park et al., 2015b) 9. TiO_2 nanotubes: • Penetration of a $Ti(OBu)_4$ solution into the BC matrix followed by hydrolysis and calcination (Wan et al., 2015)

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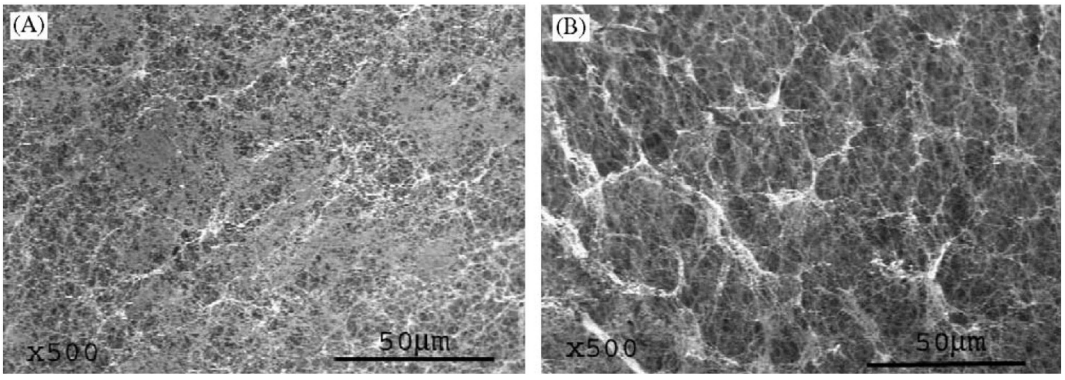

Fig. 4. Scanning electron micrograph of freeze-dried bacterial cellulose. A denser side formed at the medium/air transition zone (A), with a porous opposite layer (B). Taken from Backdahl et al. (2006).

(de Olyveira et al., 2013). Highly porous sponges can be obtained from BC fibers via emulsion freeze-drying techniques (Gao et al., 2010). Laser perforation is another technique that has demonstrated promising results in the attempt to change BC surface morphology. A pulsed CO_2 laser system achieved unidirectional and 3-D BC structuring, generating round-shaped channels of specified arrangement for the unidirectional case and orthogonally-oriented, interconnected 3-D perforation for the other case. Laser perforation causes no chemical modifications and no reduction in mechanical strength. Laser perforated BC supported the ingrowth and re-differentiation of human articular chondrocytes, which migrated through the channels into the inner matrix (Ahrem et al., 2014). Jing et al. (2011) described the attachment and proliferation of chondrogenic rat cells onto a patterned BC surface. Plasma treatment may also modify the porosity of the material; BC with greater porosity and roughness was obtained by Pertile et al. (2010) after treatment with nitrogen-containing plasma. Bottan et al. (2015) reported an improved fibroblast infiltration and new collagen deposition during *in vivo* studies on wound healing properties of BC dressings with the structured surface. The texture was transferred during the BC biofabrication process by introduction of a soft-lithographic PDMS mold in the air–liquid interface of the *Acetobacter xylinum* culture.

In addition to the porosity, the mechanical characteristics of the materials also affect cell adhesion (Vincent and Engler, 2011). The influence of the compressive modulus on cell ingrowth on a BC surface was investigated on BC samples dried using two different techniques–ambient air drying and freeze drying–and then seeded with rat mesenchymal stem cells (rMSCs). The freeze-dried BC had a softer structure than the stiffer air-dried BC. Nevertheless, both samples supported rMSC growth and differentiation—into chondrocytes in case of the softer BC and into osteocytes in the case of the stiffer material. However, the softer substrate guided the rMSC-derived chondrocytes for significantly longer time when compared to the stiffer BC. This study demonstrated the vital role of the mechanical characteristics of the substrate in maintaining cell functions (Taokaew et al., 2014).

5.2. Surface modifications

Cells respond to their external environment by adhesion when the medium conditions facilitate that process. Cells can distinguish the complex assembly of the surface chemical signals through the activation of particular receptors that provide the interaction and discrete association with the promoting groups on the surface (Spatz and Geiger, 2007). Chemical introduction of cell-responsive stimulating functionalities to polymeric substances is a principal approach for mimicking natural cell signaling unions and achieving better cell adhesion (Shekaran and Garcia, 2011).

Plasma techniques have become common procedures for surface modification of polymeric materials. These techniques change the surface properties without affecting the core attributes and introduce required alterations that improve biocompatibility. The possibility of controlling operational parameters (e.g., the choice of plasma gas, duration of exposure, and power) and the efficiency of application to materials with complex shapes, makes plasma treatment an effective tool for optimizing material biofunctionality (Chu et al., 2002; Wang et al., 2006; Yang et al., 2002). The treatment of BC with nitrogen-containing plasma led to the incorporation of N-containing functional groups and to an increase in porosity. These changes improved the adhesion of microvascular endothelial (HMEC-1) and neuroblast (N1E-115) cells. However, no improvement was seen for fibroblast (3T3) adhesion (Pertile et al., 2010). BC has also been modified with oxygen, nitrogen, and tetrafluoromethane plasmas in order to enhance cell affinity. Oxygen and nitrogen plasmas made the surface hydrophilic and promoted the incorporation of carbon–oxygen (O_2 plasma) and amide and amino (N_2 plasma) functionalities. Treatment with CF_4 plasma induced high surface hydrophobization and introduced carbon–fluoride functionalities on BC. The BC treated with CF_4 plasma exhibited significantly improved adhesion of L-929 fibroblast and Chinese hamster ovary (CHO) cells, as well as proteins from the culture media (Kurniawan et al., 2012).

Surface characteristics can also be adjusted through chemical reactions. The similarity in chemical composition of BC and plant derived cellulose fibers means that BC can be modified by the characteristic chemical reactions used for cellulose derivatization: acetylation, carboxylation, and phosphorylation (Hu et al., 2014). BC acetylation results in lower surface hydrophilicity and therefore higher initial adhesion of retinal pigment epithelium (RPE) cells compared to unmodified BC (Gonçalves et al., 2015). Chemical sulfation and phosphorylation of BC can add a charge to the surface and mimic the glucosaminoglycans of *in vivo* cartilage tissue. However, this modification did not enhance chondrocyte growth (Svensson et al., 2005). Wan et al. (2007) demonstrated that phosphorylation induced hydroxyapatite growth on a BC surface. This may be an essential response for the preparation of BC-hydroxyapatite for engineering of tissue scaffolds for bone replacement implants. Introduction of carboxyl groups to the C6 position by TEMPO oxidation revealed that surface functionalization plays an important role in the initial step of apatite nucleation (Nge et al., 2010). Impregnation of hydroxyapatite grown on a graphene oxide substrate onto a BC surface resulted in the formation of a novel material with osteoinductive potential, due to its improved biocompatibility towards human osteosarcoma MG-63 and fibroblast NIH-3T3 cell cultures (Ramani and Sastry, 2014). Introduction of aminoalkyl groups onto a BC surface through a silane chemical grafting approach led to the formation of a new material with no toxicity to human adipose-derived mesenchymal stem cells (ADSCs). The new modified material showed antimicrobial activity and could be applied in a wound healing therapy (Fernandes et al., 2013). Taokaew et al. (2015) modified BC by grafting methyl terminated octadecyl trichlorosilane (OTS) and amine terminated 3-aminopropyl triethoxysilane (APTES) in order to generate methyl

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and amine groups on the BC surface, respectively. The modifications introduced were shown to enhance hydrophobic and electrostatic interactions between normal human dermal fibroblast cells and the BC surface and therefore improved their attachment and proliferation.

5.3. Introduction of cell adhesion molecules

Cell adhesion molecules located on a surface are the mediators of cell interactions with the extracellular matrix (ECM), other cells, or soluble signaling compounds (e.g., cytokines and growth factors) (Yaseen et al., 2011). These proteins typically contain three domains: an intercellular module, a transmembrane spanning section, and an intracellular module interacting with other cells or the ECM (Ito, 2011). Functionalization of the materials with these proteins can provide biofunctionality to the materials and improve the biocompatibility for desired cell lines.

The proteins containing arginine-glycine-aspartic acid (RGD) peptides promote cell adhesion. The work of Pierschbacher and Ruoslahti (1984) specified the RGD sequence as a cellular recognition determinant. The RGD peptides have since found a wide application for functionalization of materials for biomedical applications (Hersel et al., 2003). An RGD peptide was used by Andrade et al. (2010b), who attached the cell binding sequences RGD and GRGDY to a cellulose binding module (CMB) and used it to coat BC fibers. Fibroblasts showed an improved ability to interact with the modified BC surfaces, with the RGD sequence showing a stronger effect than the GRGDY sequence. Stronger cell adhesion was seen with a protein with a single RGD sequence than with a protein with a double copy of RGD. *In vivo* implantation of RGD-functionalized BC in white merino sheep caused a mild inflammatory response, which notably decreased after 8 weeks post implantation. The implants were also considered slightly more irritating to the tissue compared to the expanded polytetrafluoroethylene material used as negative control sample. However, improved fibroblast cell adhesion and a more even cell distribution was found on the RGD modified cellulose, indicating its potential for use as a biomedical scaffold (Andrade et al., 2012). In later experiments, the same research group produced a new recombinant protein by fusing the IKVAV peptide sequence to a carbohydrate-binding module (CBM3). The proteins, stably absorbed on a scaffold, promoted an increase in adhesion and proliferation of neuronal and mesenchymal stem cells, but had no effect on the other cell lineages tested. The study highlighted the feasibility of readjusting the affinity of BC to elicit targeted cell responses (Pertile et al., 2012). A novel and sophisticated way to introduce RGD peptides onto BC with xyloglucan as a binding module was described by Bodin et al. (2007). Xyloglucan has a naturally high affinity for cellulose, which corresponds with the xyloglucan-cellulose networks in plant primary cell walls. New chemical functionalities for a cellulose surface that do not disrupt the fibers can be incorporated by binding chemically modified xyloglucan chains to cellulosic surfaces. The binding interactions, when compared to the direct chemical modifications of cellulose, fully maintain the fiber integrity and strength (Brumer et al., 2004). Xyloglucan bearing GRGDS pentapeptide conjugates were synthesized and homogeneously absorbed onto a BC surface, and these cellulose substrates provided better adhesion of human endothelial cells. Polylysine (PLL) incorporated onto BC nanofibers resulted in formation of novel fibers having similarities with collagen in terms of shape and molecular structure. Further impregnated with hydroxyapatite, BC/PLL fibers are believed to be a promising material for bone tissue replacement (Gao et al., 2011). Silk fibroin, a natural polymer consisting of two polypeptide chains is a material widely used in various medical fields (Koh et al., 2015). Its impregnation into the BC structure was shown to result in a composite with the significantly increased adhesion, spreading and proliferation characteristics towards Chinese hamster fibroblasts and L929 cells. The absence of the cytotoxicity indicates safety of BC/silk fibroin composites for biomedical applications (Oliveira Barud et al., 2015). Macrophage-stimulating protein (MSP) is known to accelerate the wound healing processes by inducing the keratinocytes proliferation and prevention of the epithelial cells apoptosis (Zhao et al., 2015). When impregnated into BC wound healing dressings, MSP was shown to promote the proliferation and migration of fibroblasts and to enhance collagen synthesis and remodeling. The detected effects were confirmed by in *in vivo* studies on burn wound models of BALB-c mice: the BC/MPC dressings accelerate wound closure and collagen synthesis of wound sites (Zhao et al., 2015). BC was also used as a carrier for bone morphogenetic protein-2 (BMP-2), a protein from the transforming growth factor beta (TGF-β) superfamily. The material demonstrated a greater biocompatibility and induced the differentiation of mouse fibroblast-like C2C12 cells into osteoblasts. When implanted subcutaneously in an *in vivo* mouse model, BC/BMP-2 showed better bone formation due to promotion of cell infiltration and osteogenesis in scaffolds. This research highlighted the potential clinical applications of BC in the treatment of bone defects and non-unions (Shi et al., 2012b).

The growth factors produced by cells are the agents that the biosignals for initiation of cell growth, differentiation, proliferation, and healing. Their wound healing properties arise due to stimulation of angiogenesis and cellular proliferation, which also affects cell inflammation and fibroblast activity (Boateng et al., 2008). The effects mentioned are essential when treating chronic wounds and the effectiveness of growth factors inclusion into the wound healing matrices was shown to accelerate the recovery of complex wounds (Gainza et al., 2015). Different growth factors have been immobilized onto BC surfaces, including basic fibroblast growth factor (bFGF), human epidermal growth factor (hEGF) and keratinocyte growth factor (KGF). These additives supported the growth of human skin fibroblasts, an important step in the wound healing process (Lin et al., 2011).

5.4. Composite preparation

Composite preparation is an effective and common way to develop novel materials with combined features of the individual components and to overcome some limitations of single materials (Heinemann, 2015). The mechanical features of BC can be complemented by the addition of biocompatible compounds to enhance the material and biological properties of the components and to extend their potential biomedical applications (Hu et al., 2014). There are a number of natural and synthetic polymers as well as some inorganic compounds which may be used for these purposes.

Among many other natural polymers, collagen is commonly used in various biomedical applications. Collagen structure closely matches living tissues; it creates a natural extracellular environment that promotes cell attachment and proliferation (Zeugolis and Raghunath, 2011). BC/collagen composites were prepared by immersing wet BC pellicles into collagen solutions. SEM images showed that collagen molecules penetrated into the BC structure and formed multilayers, resulting in an increased Young's modulus and decreased elongation at the break point. The composite materials promoted good adhesion and proliferation of 3T3 fibroblasts cell and showed much better cytocompatibility than pure BC. This new material was reported to have the potential for use as a wound dressing material or artificial skin (Zhijiang and Guang, 2011). Saska et al. (2012) prepared esterified BC/collagen membranes as a new biomaterial for bone tissue engineering, but this composite material did not support better viability of osteogenic cells when compared to non-modified BC. The total protein content and alkaline phosphatase activity indicated that the composite material should allow the *in vitro* development of the osteoblastic phenotype. Gelatin has been also used to improve BC compatibility. It is a heat-denaturated collagen, and its favorable properties, such as biocompatibility, promotion of cell adhesion and proliferation, low immunogenicity, and low cost, make it ideally suitable as a biomaterial for medical applications (Keenan, 2000). When generated *in situ* on BC fibers, gelatin at concentrations below 0.25% wt forms spherical

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particles on the surface of the BC nanofibrils. At higher concentrations, gelatin coats the fibers, preserving the original BC 3-D network structure, which can be fully filled with gelatin at gelatin concentrations above 0.5 wt.%. The composites show better bioactivity towards fibroblast NIH3T3 cells and support better adhesion and proliferation (Wang et al., 2012b). Similar results were demonstrated by Kim et al. (2010a) for the biocompatibility of BC/gelatin composites and 3T3 fibroblast cells. Kim et al. (2010b) also used chitosan as a second component to improve BC composite biocompatibility. Chitosan is known for its antimicrobial activity, biocompatibility, biodegradability and mechanical stability properties (Jiang et al., 2014). A BC/chitosan composite material showed a better biocompatibility with 3T3 fibroblast cells when compared with pure BC (Kim et al., 2010b). Higher fibroblast L929 viability and the absence of cytotoxicity were also observed by Lin et al. (2013a). The adhesion of human skin keratinocytes and fibroblasts was confirmed by Kingkaew et al. (2014). Gonçalves et al. (2015) reported better adhesion of retinal pigment epithelium (RPE) cells. Low molecular weight chitosan impregnated into BC matrix promoted the growth of human skin cells. A novel BC-chitosan/heparin composite prepared by Wang et al. (2012a) with the aim of improving blood compatibility was suitable for MC3T3-E1 fibroblast cell proliferation and ingrowth. A natural mixture of phospholipids and neutral lipids known as lecithin has been also used to improve BC interaction with cells (Zhang et al., 2015). The novel composite showed significantly better support for proliferation of breast cancer cell line MDA-MB-231 as compared to neat BC. Anionic polymers such as alginate are also utilized for BC modification. Alginate forms biocompatible and non-toxic hydrogels that are widely used in wound healing, drug delivery, and tissue engineering. Its relatively low cost and its tendency for mild gelation in the presence of various divalent cations (e.g., Ca^{2+}, Ba^{2+}) creates a broad spectrum for alginate utilization (Skjåk-Bræk and Draget, 2012). *In vitro* studies on human keratinocytes (HaCat) and gingival fibroblasts (GF) demonstrated that BC/alginate sponges supported cell proliferation and could be used as wound dressings, especially when taking into account their structural stability and good tear resistance (Chiaoprakobkij et al., 2011). Polyhydroxyalkanoates (PHAs) are polyesters of 3-, 4-, 5-, and 6-hydroxyalkanoic acids and are produced under nutrient-limiting conditions by different bacterial species. They are biodegradable polymers with good biocompatibility and so are of interest as materials for biomedical applications. The medium chain length poly(3-hydroxyoctanoate) [P(3HO)] was used in a new composite with acetylated BC. The composite showed improved biodegradability and mechanical properties and also supported the proliferation of human microvascular endothelial tissue culture cells (HMAC-1s). The material can be used for the fabrication of biodegradable stents (Basnett et al., 2012). Composites of BC with poly(3-hydroxybutyrate) [PHB] and poly(3-hydroxybutyrate-co-4-hydroxybutyrate) [P(3HB-co-4HB)] also supported the adhesion of Chinese Hamster Lung (CHL) fibroblast cells and are under consideration as bioactive materials in various biomedical applications (Zhijiang et al., 2011, 2012). The combinations of the

Synthetic polymers have also been used for composite preparation. A number of synthetic polymers are in active use in biomedical applications, and especially in wound treatment. Fibers obtained by electrospinning of polylactic, polyacrylic, and polyglycolic acids, polyethylene glycol, polymethacrylate, polyvinyl alcohol, polycaprolactone etc., are able to mimic the extracellular matrix and thereby facilitate the wound healing process (Mogosanu and Grumezescu, 2014). Poly(ethylene glycol) has good biocompatibility and is used in a wide range of applications in various areas of medical industry. It functions as a conjugating ligand for drug delivery systems to decrease uptake by the reticuloendothelial system and it prolongs blood residence, decreases degradation by metabolic enzymes, and reduces protein immunogenicity (Gref et al., 2012; Kolate et al., 2014). In tissue engineering, it is used as a coating for implants (Tejero et al., 2014) and as a coating for treatment of wounds and burns (Mogosanu and Grumezescu, 2014). A foam-liked BC/PEG composite was prepared by soaking BC with an aqueous PEG solution followed by freeze-drying. The biocompatibility of the composite was estimated by cell adhesion studies using 3T3 fibroblast cells, which revealed a better cell adhesion and proliferation profile for the modified material than for pure BC (Cai and Kim, 2009; Kim et al., 2010a). BC/Poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogels are non-cytotoxic to human adipose-derived mesenchymal stem cells (ADSCs), which spread well, correctly adhered, and proliferated to fully cover the composite membranes. The increases in thermal stability, storage tensile modulus, and swelling ratios of this hydrogel indicate a potential for its application as a dry dressing (Figueiredo et al., 2013).

Some inorganic materials may be interesting for the fabrication of materials for specific applications. Thus, carbon nanotubes (CNT) have been tested as a functional material for bone regeneration scaffold preparations due to their property to promote bone tissue formation through direction of mesenchymal stem cells differentiation (Xu et al., 2015). A study on the bone regeneration efficacy of the BC/CNT composites has demonstrated that these materials result in better bone regeneration of the scull bone tissue of the ICR mice as compared to the pure BC. Moreover, *in situ* impregnation of the CNT nanoparticles into BC during the biosynthesis lead to a homogeneous distribution of CNT's throughout the microporous structure of the scaffold. This explains the higher performance of the composite in comparison with the material obtained by soaking of BC in CNT solution (Park et al., 2015b). If CNT will also have negative effects induced by their nanostructure still remains to be elucidated. TiO_2 nanotubes are known in the field of biomedical engineering as material mimicking the dimensions of natural bone constituent components (von Wilmowsky et al., 2009). Its impregnation into the BC matrix resulted in the composites with improved mouse fibroblast and human osteoblast cell lines growth and proliferation degrees compared to the tissue culture plate control. The novel scaffold material is expected a step forward in bone tissue engineering scaffold research (Wan et al., 2015).

6. Introduction of antimicrobial activity into bacterial cellulose dressings

BC itself does not have antimicrobial properties to prevent infection ingrowth and represents just a physical barrier against bacterial invention (Czaja et al., 2006b). This may reduce its effectiveness as a treatment for highly contaminated wounds. Additional modifications are needed to add antimicrobial activity to BC-based wound dressings. Several approaches can be used to introduce antimicrobial properties into BC material. Impregnation of antimicrobial agents into the BC microporous structure provides slow release of the drug into the colonized wound and long-lasting action against microorganism growth (Moritz et al., 2014; Wu et al., 2014a). Generally, the impregnation of additional components into BC structure is not an intricate process. The large surface area and presence of hydroxyl groups capable of intermolecular and chemical interactions mean that BC has a great adsorption capacity towards both large biomacromolecules, such as proteins or polysaccharides, and to smaller molecules, such as quaternary ammonium compounds or nanoparticles (Chen and Huang, 2015; O-Rak et al., 2013; Ougiya et al., 1998; Zhang et al., 2015). However, chemical modifications can provide BC with additional adsorption capacity for desirable antimicrobial substances. For example, phosphorylation of BC results in better protein adsorption. All tested proteins (e.g., lysozyme, hemoglobin, myoglobin and albumin) showed pronounced adsorption rates onto an anionic phosphorylated BC surface under pH conditions lower than their isoelectric points (Oshima et al., 2011). Lin et al. (2015a) showed an increase in adsorption quantity of bovine serum albumin with the increase of carboxymethyl functional groups in the BC substrate. This attribute accentuates the attractiveness of using BC in biomedical applications as a material capable of carrying additional functionalities essential for specific wound occurrences. Chemical

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modification of the BC surface is an alternative method for activating the structure against immune response-inducing bacteria without the addition of exogenous components. Introduction of quaternary ammonium groups to the cellulose chain imparts antimicrobial activity analogous to quaternary ammonium antiseptics and disinfectants (Förch et al., 2014). Cell adhesion functional sites can also be incorporated into cellulose, resulting in capture of bacterial cells at the activated surface without their actual destruction. The microorganisms can be irreversibly eliminated during the dressing exchange procedure (Butcher, 2011a). This alternative approach to bacterial control has recently gained heightened attention as a promising way to reduce colonization level without inducing inflammatory responses due to dead cell debris (Butcher, 2011b). These possibilities, advantages and drawbacks of introducing antibacterial properties into BC are summarized in Table 5.

6.1. Impregnation of bacterial cellulose with antimicrobial agents

The choice of eligible antimicrobial agent is influenced primarily by its specificity and efficacy against likely contaminants and pathogens. The ideal antimicrobial should be inexpensive, non-toxic, non-carcinogenic, and fast acting and should not interfere with the wound healing process (Butcher, 2012). Antiseptics are more preferable than antibiotics as they are less likely to lose their effectiveness due to bacterial resistance (Lipsky and Hoey, 2009). Attempts to improve the antimicrobial efficiency of BC-based dressings have involved the impregnation of numerous organic and polymeric substances as well as inorganic materials bearing antimicrobial activity into the BC matrix. The topical antiseptics utilized for BC modifications have included biological and synthetic polymers with antimicrobial activity (Figueiredo et al., 2015; Jiang et al., 2014), antimicrobial peptides (Basmaji et al., 2014), cationic antiseptics (e.g., quaternary ammonium compounds and biguanides) (Kukharenko et al., 2014; Serafica et al., 2010), antibiotics (Lacin, 2014), and inorganic compounds (e.g., metal or graphene nanoparticles and metal oxides) (Shao et al., 2015a,b; Ul-Islam et al., 2014). The general principle of bacterial inactivation by different antimicrobial active substances is similar — the disruption of the bacterial cytoplasmic membrane. Fig. 5 illustrates the general mechanism of action of various antimicrobial active compounds (Förch et al., 2014; Gilbert and Moore, 2005; Zasloff, 2002). The chemical composition of the quaternary ammonium compounds, cationic polymers and antimicrobial peptides plays a key role in the disruption of the bacteria's' membrane phospholipid bilayer. These molecules carry positively charged groups and have a strong affinity for the negatively charged cell walls of bacteria. In addition, the molecules bear apolar alkyl chains and spacers, which ensure the mobility of the cationic group (Gilbert and Moore, 2005). Progressive adsorption of the positively charged groups onto acidic phospholipids in the membrane bilayer leads to the disruption of the membrane protein function due to the solubilization

Table 5
Summary table of possible methods for introducing antimicrobial properties into bacterial cellulose (BC) based wound dressings.

Modification method	Inclusion of antimicrobials into the BC matrix	Surface hydrophobization	Fiber modification through covalent binding of cell adhesion sites
Principle of action	Slow release of bacteria-destroying particles into the wound	Bacterial binding to the dressing due to cell-surface hydrophobicity effect and their irreversible elimination during dressing changes	Covalently attached active sites provide permanent antimicrobial activity and bacteria binding capacity
Pros	Most effective method to heal colonized wounds Least expensive method for industrial production	Reduced endotoxin release due to the absence of dead cell debris Constant activity over time	Reduced biocide release Constant activity over time
Cons	Substrate performance lost over time Bacterial resistance to the active components	Lowest efficiency between all types of antimicrobial dressings; not applicable for contaminated wound treatment	Expensive and comprehensive for industrial production Lower effectiveness compared to non-attached antimicrobials
Examples	1. Biological and synthetic polymers with antimicrobial activity: • Chitosan (Kingkaew et al., 2014; Lin et al., 2013a) • Poly(2-aminoethyl methacrylate) (Figueiredo et al., 2015) 2. Antimicrobial peptides (Basmaji et al., 2014) 3. Cationic antimicrobial agents: • PHMB (Serafica et al., 2010) • Polyhexamethylen guanidine hydrochloride (Kukharenko et al., 2014) • Benzalkonium chloride (Wei et al., 2011) • Octenidine dihydrochloride (Moritz et al., 2014) 4. Antibiotics: • Chloramphenicol (Lacin, 2014) 5. Inorganic nanoparticles: • Ag (Wu et al., 2014a) • ZnO (Katepetch et al., 2013) • TiO_2 (Limaye et al., 2009) • Montmorillonite (Ul-Islam et al., 2012a) • Graphene oxide (Shao et al., 2015b)	Treatment with dialkylcarbamoyl chloride (DACC) (Schütz and Schultz, 2014) Treatment with alkyl ketene dimer (Bjornberg and Smith, 2014)	Introduction of amino groups: • Chemical grafting of 3-aminopropyltrimethoxysilane (Fernandes et al., 2013) • Plasma treatment (Pertile et al., 2010) Binding of RGDC peptides/gentamicin (Rouabhia et al., 2014)

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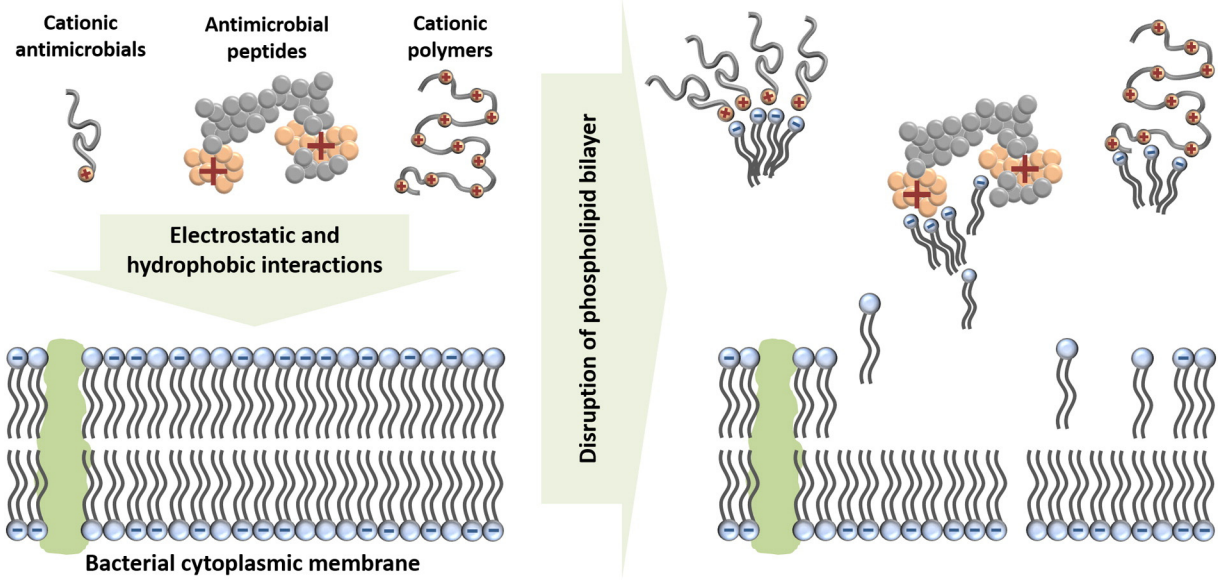

Fig. 5. Representation of the antibacterial mode of action of antimicrobial active substances carrying cationic active group and apolar domain leading to bacterial cytoplasmic membrane disruption (Förch et al., 2014; Gilbert and Moore, 2005; Zasloff, 2002).

of phospholipids and proteins into cationic antimicrobial/phospholipid micelles, which also causes cell lysis. The alkyl chain that is part of the antimicrobial structure plays an essential role in this "hole-poking" mechanism due to its high lipophilicity and causes damage to cell wall integrity (Förch et al., 2014; Zasloff, 2002). The bactericidal mechanism of antibiotics and metal nanoparticles follows a more complicated process and is discussed in corresponding paragraphs.

6.1.1. Biological and synthetic polymers with antimicrobial activity

Some natural polymers promote wound healing due to their favorable antimicrobial effectiveness. Impregnation of these biopolymers into the microporous BC structure could create composites with enhanced activity against pathogenic microorganisms. Chitosan, due to its cationic nature and the presence of amino groups in its structure, shows a wide-spectrum activity against Gram-positive and Gram-negative bacteria. It also stimulates the production of type III collagen in wound areas and promotes the migration and proliferation of fibroblasts, the principle cells that accelerate the proliferation healing stage (Jiang et al., 2014). BC membranes carrying chitosan on their surfaces demonstrated a marked inhibition of growth of Gram-positive *Staphylococcus aureus* (*S. aureus*) and Gram-negative *Escherichia coli* (*E. coli*) bacteria. A comparison of the wound healing by these composite membranes to that of commercial hydrocolloid dressings and transparent film dressings in a Sprague Dawley rat model revealed that the BC/chitosan membranes promoted a greater average wound contraction ratio and tissue regeneration, indicating their good potential in wound treatment (Lin et al., 2013a). The absorption capacities, release rate, and antimicrobial activity of BC films modified with chitosan of different molar weights were investigated by Kingkaew et al. (2014). Regardless of its molar weight, chitosan could penetrate into the BC fibril network and fill the pores, although the adsorption capacity was highest for the lowest molar mass chitosan. The release ratio decreased with increasing chitosan molar weight. The chitosan concentration was an important factor contributing to the antibacterial property of the material. Films with a higher chitosan concentration (i.e., the lowest chitosan molar mass) showed better antimicrobial abilities against *S. aureus* and *Aspergillus niger* (*A. niger*). Post-synthetic impregnation of chitosan was more efficient than *in situ* preparation of a BC-chitosan composite during the biosynthesis process. The same working group also reported the absence of significant antimicrobial activity for *in situ* prepared BC/chitosan films, apparently because of the small amount of chitosan that was impregnated the BC structure (Phisalaphong and Jatupaiboon, 2008).

Figueiredo et al. (2015) performed *in situ* radical polymerization of 2-aminoethyl methacrylate (AEM) into the BC network without and in the presence of *N*,*N*-methylenbis(acrylamide) (MBA) used as cross-linker. The polymer carrying ammonium groups was shown to fill the porous BC structure completely. BC/poly-AEM materials were demonstrated to have antimicrobial activity against *E. coli*. Cross-linked BC/poly-AEM/MBA samples, however, did not show antimicrobial properties, which was attributed to the reduced diffusion of the bacteria into the cross-linked sample and therefore the lowered contact of the microorganisms with the ammonium groups (Figueiredo et al., 2015).

6.1.2. Antimicrobial peptides

Membrane-active antimicrobial peptides are currently of high medical interest as a promising source of novel antimicrobials effective against multiresistant pathogens. In general, these peptides act by physical disruption of cell membrane integrity. They play essential roles in the adaptive and innate immune system and show very little tendency to elicit resistance. Therefore, they may represent a new generation of effective antimicrobials (Van 'T Hof et al., 2001). To the best of our knowledge, only one publication has reported on the incorporation of antimicrobial peptides into BC. A novel peptide was produced by symbiotic culture using polyhexanide biguanide and green tea. This resulted in a 3-D network of novel BC/polyhexanide biguanide nanofibers that showed great potential for management of chronic wounds (Basmaji et al., 2014). Some publications were related to synthesis of these peptides on plant-derived cellulose (Hilpert and Hancock, 2007) and their impregnation onto a chemically modified cellulose surface (Hilpert et al., 2009). The unique properties of antimicrobial peptides may lead to further investigations in this direction.

6.1.3. Cationic antimicrobial agents

Cationic antimicrobials, such as quaternary ammonium compounds, biguanides, and bisbiguanides have been actively used for decades in medical and everyday life as routine antiseptics and disinfectants. One of the most actively utilized biguanide polymer is poly(hexamethylenebiguanide) hydrochloride (PHMB) (Gilbert and Moore, 2005). Its long-term and wide use as a routine antiseptic in first aid, surface, and linen disinfection has led to the production of commercially available dressings based on BC impregnated with PHMB as an

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active substance to impart antimicrobial or inhibitory activity for use in chronic and burn wound management (Serafica et al., 2010). The effectiveness of cationic antimicrobials against Gram-negative bacteria is limited, however. This is because of the additional outer membrane surrounding the cytoplasmic membrane in these species. The Gram-positive species have only a single protective layer and can be easily disrupted by antimicrobial agents. The additional layer in the Gram-negative bacteria contains liposaccharides and porins with narrow restrictive channels that act as barriers to foreign molecules and retard their binding to the cytoplasmic membrane (Nikaido, 1994). The performance of each individual antimicrobial agent used for impregnation into a BC wound dressing therefore has to be tested beforehand.

Other cationic surfactants in addition to PHMB have been used to introduce biocidal activity to BC-based wound dressings. BC dressings with polyhexamethylene guanidine hydrochloride showed great efficacy against a broad range of bacteria strains, such as multidrug resistant *S. aureus* and *Klebsiella pneumonia* (*K. pneumonia*), the phytopatogenic *Xanthomonas campestris* (*X. campestris*) and *Pseudomonas syringae* (*P. syringae*), as well as yeast (Kukharenko et al., 2014). Wei et al. (2011) tested the activity of freeze-dried BC film soaked in benzalkonium chloride solution against Gram-negative *E. coli* and Gram-positive *S. aureus* and *Bacillus subtilis* (*B. subtilis*) bacteria. The benzalkonium chloride solution itself showed antimicrobial activity against all three tested bacteria, but the modified BC film had little effect on the growth of *E. coli*. Its effects on the Gram-positive bacteria were significantly better. These antimicrobial BC dry films are prospective commercial products for acute trauma treatment. Moritz et al. (2014) used octenidine dihydrochloride for the post-synthetic modification of BC and showed that the modified material had significant antimicrobial activity against *S. aureus* even after a 6 month storage period and exerted only minimal cytotoxic effects on human keratinocytes.

6.1.4. Antibiotics

BC dressings can be also functionalized with some broad-spectrum antibiotics. Chloramphenicol is the drug of choice for treatment of a wide range of bacterial infections. Its antimicrobial mode of action differs from those of the substances carrying the cationic groups (Fig. 5). It binds to the 50S ribosomal subunits blocking some fundamental ribosomal functions (Xaplanteri et al., 2003). Chloramphenicol (CAP)-loaded dialdehyde (DABC) and non-oxidized BC membranes were investigated in terms of their antimicrobial efficiency against *E. coli*, *S. aureus*, and *Streptococcus pneumoniae* (*S. pneumoniae*). The drug loading capacity of DABC was quite low, but both membranes showed a prolonged antimicrobial effect against the tested bacteria. The adhesion and proliferation of fibroblasts cell line L929 on the CAP/DABC membrane were noticeably higher than that on the non-oxidized BC. This study highlighted the potential for use of newly developed CAP/-DABC dressing materials in wound treatment (Lacin, 2014).

6.1.5. Inorganic compounds

In general, inorganic nanomaterials play an important role in antibacterial applications due to their large surface area and the properties imparted by their particle shapes (Moghimi and Farhangrazi, 2014). Nanoparticles (NPs) have a tendency to aggregate and uncontrollable release of ions and potential cytotoxicity are the major disadvantages in their use. The recent progress in fabrication of polymer matrices impregnated with antibacterial active NPs is now attracting great interest in research in this area. (Rai et al., 2009) In particular, nanosilver exhibits a broad-spectrum antibacterial activity that is more effective than bulk silver materials. Silver has an extensive history of implementation as an antimicrobial agent. The antibacterial effect of silver cations is the result of its interaction with negatively charged peptidoglycans in the bacteria cell wall. This causes the misfolding of proteins, followed by disabling of oxygen metabolizing enzymes and bacterial death. Silver can also diffuse through the membrane cell wall and interact with DNA, as well as with thiol groups of enzymes or important respiratory proteins. This broad mode of action means that silver has antimicrobial activity against both Gram-positive and Gram-negative bacteria (Guo et al., 2013).

Several methods have been developed for the incorporation of silver NPs into a BC matrix. In general, nanoparticles can be incorporated by direct diffusion of previously synthesized NPs (Zou et al., 2008) or added to a synthetic mixture (Geng et al., 2011). Another possibility is the *in situ* synthesis of the nanoparticles on the BC matrix. Direct adsorption is a relatively easily controlled process; however, it can be disrupted by the formation of aggregates (Zou et al., 2008). Silver NP formation can be observed through the color change from yellow to dark brown, which is attributed to the surface plasmon resonance of conducting electrons on the surface of the silver nanoparticles (Pourali et al., 2014). In contrast, the *in situ* synthesis approach is more advantageous in terms of the formation of uniformly distributed particles, although carrying out the procedure of ion loading may be more complicated. Table 6 shows an overview of the different mechanism for *in situ* formation of silver nanoparticles on a BC matrix.

Another way to introduce slow-releasing Ag ions to the dressing structure is by impregnation of silver-containing substances such as silver sulfadiazine (SSD). This antimicrobial agent is an active component used in burn and wound therapy due to its wide spectrum of antibacterial and antifungal action. The mechanism of its action is related to ionization and silver ion release (Dellera et al., 2014). SSD exerts its cytotoxic effects by interfering with both bacterial as well as the host cells, but these effects can be minimized by the control of ion release (Piatkowski et al., 2011). The association of SSD with biopolymers such as BC may represent an auspicious approach for creating non-toxic biomaterials with favorable wound healing and antimicrobial features. BC/SSD membranes show significantly improved activity against *Pseudomonas aeruginosa* (*P. aeruginosa*), *E. coli*, and *S. aureus* when compared with pure BC, but the modification did not affect biocompatibility as determined by the growth of epidermal cells and fibroblasts. Therefore, BC/SSD membranes are promising antimicrobial wound dressings for burn treatment (Luan et al., 2012).

A BC composite with ZnO nanoparticles also showed antimicrobial activity against *E. coli* and *S. aureus*. The composite was fabricated by ultrasonic assisted *in situ* precipitation of zinc oxide onto the BC surface, resulting in the formation of nanocrystalline particles without affecting the 3-D structure of the BC substrate (Katepetch et al., 2013). Bacterial growth inhibition was observed when regenerated BC was used as a substrate for ZnO NPs. The inhibitory effect was shown to be dependent on the NPs' final concentration in the product (Ul-Islam et al., 2014). Recently, Xiong et al. (2014) reported that the morphology of *in situ* precipitated ZnO NPs is controlled by BC hydroxyl groups due to electrostatic interactions with Zn^{2+}. In the presence of BC the particles are tending to form flower-like aggregates, in contrast to spindle-shaped ZnO NPs formed without BC. Photocatalytic disinfection using photoactive substances is based on the capability of the active molecules to form free radicals when exposed to incident UV light. Radicals undergo secondary reactions with organics in air and water thereby providing antimicrobial activity (Blake et al., 1999). The effectiveness of BC-based antimicrobial dressings with impregnated TiO_2 nanoparticles was reported against some strains of Gram-positive and Gram-negative bacteria as well as against yeast colonies (Khan et al., 2015; Limaye et al., 2009). The bactericidal mechanism of TiO_2 nanoparticles is complicated and represents the combination of various affection mechanisms, such as direct contact of the metallic ions with cell membrane causing its damage and generation of reactive oxygen species (ROS) — hydrogen peroxide, superoxide and oxygen free radicals which also cause the damage of surface of the phospholipid layer of the outer membrane of microorganisms (Khan et al., 2015). In addition, it was demonstrated that ROS generated are able to permeate the cell wall and disrupted the bacterial life cycle by oxidation of thiol groups of various amino acids present in bacterial cells (Blake et al., 1999; Khan et al., 2015).

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Table 6
Overview of the possible approaches for *in situ* generation of silver nanoparticles on a bacterial cellulose (BC) matrix.

Method of *in situ* formation of Ag NPs	Efficiency against bacterial lines	Average size, nm	Comments	Refs
1. Synthesis of Ag NPs on BC/AgCl matrix by *G. xylinum*	*E. coli*, *S. aureus*	17.4	Additional formation of AgCl NPs (34.2 nm)	Liu et al., 2012
2. Reaction of Tollens' reagent with BC	*S. aureus*	10-30	Promotion of wound healing in a rat wound model	Wu et al., 2014a
3. Chemical reduction of Ag^+ with:				
• $NaBH_4$	*S. aureus, B. subtilis, C. albicans, E. coli*	11.3; 6.5; 5.5	Particle size depends on $AgNO_3/NaBH_4$ molar ratio Antimicrobial activity depends on the Ag concentration	Maneerung et al., 2008; Shao et al., 2015a
• Triethanolamine (TEA)	*E. coli*, *S. aureus*	8; 10; 15	Particle size depends on the amount of TEA	Barud et al., 2011; Pinto et al., 2009
• UV radiation	*B. subtilis*, *S. aureus*	26.94 ± 1.99	–	Pinto et al., 2009; Yang et al., 2015
• Hydrazine with gelatin/polyvinylpyrrolidone	–	40-60/80-100	Gelatin or polyvinylpyrrolidone are used as colloid protectors	de Santa Maria et al., 2009
• Hydroxylamine with gelatin/polyvinylpyrrolidone	–	30-50/70-100	–	de Santa Maria et al., 2009
• Ascorbic acid	–	50-70/70-100	–	de Santa Maria et al., 2009
• DMSO	*E. coli*	–	–	Berndt et al., 2013
• Thermal reduction	–	13,1	Reaction with aminoalkane-grafted BC; Reaction of Ag salt of pre-oxidized BC; particle size can be control by adjusting the degree of substitution of C6 carboxylate groups	Ifuku et al., 2009
	E. coli, S.aureus, P. aeruginosa	40-60	Autoclaving at 0.103 MPa pressure, 121 C for 10 min; reduction by cellulose end aldehyde and alcohol groups	Shao et al., 2015b
• Polydopamine	*E. coli, B. subtilis*	30-50	Reaction with iron-based magnetic BC coated with polydopamine	Sureshkumar et al., 2010

Nanoparticles other than metal NPs also show biocidal activity. Montmorillonite (MMT), a clay mineral, has a broad spectrum of biomedical applications. MMT and its products are non-toxic antimicrobial agents with high efficiency against both Gram-positive and Gram-negative bacteria (Hong and Rhim, 2008). MMT has protective properties and promotes accelerated healing. BC-based composites impregnated with pure MMT, as well as with Na-, Ca-, and Cu-modified MMTs, were synthesized and characterized in terms of their antimicrobial activity in order to determine their suitability for use as dressing materials and artificial skin. All the composites showed antibacterial activity against *S. aureus* and *E. coli*, but the effectiveness was directly correlated with the amount of MMTs. The BC/Cu-MMT composites more strongly inhibited growth of the tested bacteria when compared with the other materials. These composites could be used as novel biomaterials for wound dressings and regeneration materials (Ul-Islam et al., 2012a). BC composites with graphene oxide nano sheets proved excellent antimicrobial activity against *E. coli* and *S. aureus* (Shao et al., 2015b).

6.2. Surface hydrophobization to improve antimicrobial activity

The concept of direct release of antimicrobial agents into a wound and *in situ* destruction of infection raises questions about effectiveness. The main opposition is related to the presence of bacterial cell debris after the destruction of pathogenic organisms. The positive effect of reduced exotoxin levels after the reduction of the bacterial load is likely to be overshadowed by endotoxin release after bacterial death and disruption, together with other inflammatory responses to cell debris. Therefore, the optimal reduction of bacterial number and proliferation rates during the long-term wound restoration process should avoid bacterial death and wound contamination (Butcher, 2011a). This new paradigm of wound dressing antimicrobial effectiveness consists of binding an immune response-inducing bacterium or fungus to the wound dressing, followed by its irrevocable elimination by the dressing change (Butcher, 2011b). The adhesion forces of different natures, including physico-chemical interactions as well as mechanical capture of the bacteria within the substrate matrix, may confine the microorganisms to the dressing core or on its surface (Hjertén and Wadström, 1990). The best studied property of the microbial cell surface with regard to its adhesion to other surfaces is cell-surface hydrophobicity (CSH). The fundamental driving force of CSH adhesion is believed to be the assembly of all structural and physico-chemical interactions affecting microbial adhesion rather than a single entropy factor (van der Mei et al., 1998). Cellulose-based wound dressings coated with a hydrophobic layer can be fabricated by treatment with dialkylcarbamoyl chloride (DACC) or alkyl ketene dimer. The dressings reported to contain at least one hydrophobic antimicrobial layer and at least one absorbent layer consisting of a water-containing hydrogel. Modified dressings demonstrated antimicrobial and antifungal activity (Bjornberg and Smith, 2014; Schütz and Schultz, 2014).

The antimicrobial efficiency of these dressings with hydrophobic surfaces is, however, still under consideration. The varieties of commercially available wound dressings with and without hydrophobic surfaces were examined for their capacities to eliminate bacteria such as methicillin-resistant *S. aureus* (MRSA) and *P. aeruginosa*. Antibacterial effectiveness of the DACC-coated dressings was demonstrated to be slightly lower when compared to non-coated polyurethane dressings (with and without silicone), and significantly lower when compared to a dressing releasing silver ions (Braunwarth and Brill, 2014). Although bacterial elimination by a hydrophobic surface was confirmed, it can be improved by combination of this passive effect with the use of active ingredients such as, for example, silver ions or other antimicrobials. This strategy may significantly improve the colonized wounds healing rates, yet the strategy proving investigations are still missing.

6.3. Alternative surface modifications for introducing antimicrobial functionalities

Generally, antimicrobial surfaces can be generated by incorporation of antimicrobial sites by covalent or non-covalent interactions with the surface. The efficiency of impregnation of antimicrobial additives non-covalently bound to the surface has already been discussed. The major disadvantage of this approach is the continuous drop of the concentration of active component due to its constant release from the dressing to the wound environment and therefore loss of the dressing antimicrobial performance (Butcher, 2011a). Immobilization of antimicrobial groups or polymers bearing antimicrobial sites onto the substrate

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surface via chemical reactions can generate materials with permanent antimicrobial activity. The lack of biocide release also prevents wound contamination with dead bacterial cells and release of endotoxins.

The presence of amino groups along the polymer chain imparts a natural antimicrobial activity to some biopolymers, such as chitosan (Jiang et al., 2014). This intrinsic antimicrobial property can be mimicked by introducing nitrogen-containing groups to the BC surface through chemical interactions (Fernandes et al., 2013). Antibacterial BC membranes have been prepared using a silane chemical grafting approach by impregnation of 3-aminopropyltrimethoxysilane onto the surface of BC nanofibrils. The membranes were lethal against *S. aureus* and *E. coli* but were nontoxic to human adipose-derived mesenchymal stem cells. The modified material is a potential candidate for various biomedical applications (Fernandes et al., 2013). The same silane grafting approach was used to covalently bind RGDC peptides/gentamicin to the surface of a BC membrane network. The membranes were bactericidal against Gram-positive *Streptococcus mutans* (*S. mutans*) and biocompatible with human dermal fibroblasts, indicating suitability as a material for wound healing and drug delivery systems (Rouabhia et al., 2014). The silane grafting approach is generally a promising tool to introduce functional groups of different kinds to the BC surface. It was demonstrated by Hettegger et al. (2015), the grafting of azidoalkyl groups onto wet BC can be performed under mild, environmentally friendly conditions. The modification does not affect BC fibrillar structure; the fibrils on the surface were shown to be covered by siloxane together with 3-D polysiloxane network, providing accessibility and proper reactivity of the azide functional groups towards further click chemistry derivatization reactions. An elegant and easy method for introducing triazine derivatives containing quaternary ammonium and multi-cationic benzyl groups was described by Hou et al. (2009). Cellulose samples were kept in 2,4-bi[(3-benzyl-3-bimethylammonium)propylamino]-6-choro-1,3,5-triazine chloride (BBCTC) alkali solution and then washed to remove unfixed compounds. The modified fabric demonstrated excellent antibacterial properties against *S. aureus*. Cationic monomers carrying quaternary ammonium groups were also directly polymerized onto a cellulose surface. As an example, poly 2-(dimethylamino)ethyl methacrylate grafted onto cellulose filter paper via RAFT polymerization imparted bactericidal activity against *E. coli* to the modified fibers (Roy et al., 2007). Another approach to adjust cellulose functionality is grafting of β-cyclodextrin onto the fibers. This compound, a cyclic oligosaccharide, readily forms host-guest complexes with various small molecules, including antibiotics and antimicrobial agents and grafting reactions between cellulose and β-cyclodextrin have been successfully performed. The inclusion of ciprofloxacin hydrochloride as a model antibiotic in cellulose fibers resulted in strong antimicrobial activity against *E. coli* and *S. aureus* (Dong et al., 2014). A novel approach to introduce different kinds of active substances, such as antimicrobial agents, disinfectants, or vitamins, was proposed by Rosenau et al. (2005). Organic molecules were bounded to the cellulosic fibers via monochlorotriazinyl anchor group. Modified fibers performed slow controlled release of the active agents, activated by contact with atmospheric moisture. These reactions were optimized on plant-derived cellulose fibers or fabrics, but the same approaches are applicable for modification of the structurally identical BC.

Surface properties can also be altered by plasma techniques. Nitrogen-containing plasma treatments added N-containing functional groups to the BC surface (Pertile et al., 2010). The presence of $^{*}C–N^{+}$ on the filter paper surface after the treatment with cold ethylene diamine plasma also imparted biocidal characteristics against Gram-positive *S. aureus* and Gram-negative *K. pneumonia* bacteria (Jampala et al., 2008). BC surfaces with anchored amino groups can be further modified with other anti-bacterial agents. For example, morphologically different bacteriophages, active against different types of bacteria (*E. coli*; *Salmonella enterica* (*S. enterica*), *Listeria monocytogenes* (*L. monocytogenes*), and *Shigella boydii* (*S. boydii*)) showed an affinity for silica surfaces, and particularly to those organically modified with amines or carboxylates and having a greater surface charge (Cademartiri et al., 2010).

7. Bacterial cellulose water holding capacity and water release rate

The ability of the wound dressing to absorb high amounts of the liquid is particularly important when treating the wound producing high amount of exudate. Excessive exudate may result in the separation of the tissue layers and therefore delay healing process (Hedlund, 2007). Its proper absorption is an important feature of the modern wound dressings. However, the balance between liquid absorption and donation should be preserved; no dehydration of the wound surface should take place for the fast and successful sore recovery (Davidson, 2015). Therefore the material liquid migration characteristic has to be taken into account when producing wound dressing systems. The water holding capacity (WHC) and water release rate (WRR) values of BC differed when the physico-chemical and structural characteristics were modified. Alteration of the surface area and pore size distribution caused the most significant changes (Ul-Islam et al., 2012b). Another possibility for adjusting the WHC and WRR parameters is to introduce additional hydrophilic components to the BC structure.

7.1. The influence of pore size distribution and fiber morphology

The decrease of BC WHC values is attributed to the formation of more compact structure with denser fibril disposition, and therefore reduced pore volume and surface area (Wang et al., 2012b). This fiber organization reduces available space and amount of trapping sites for penetrating water molecules. On the other hand, the denser microfibrils result in a higher amount of water held back in the system due to the hydrogen bonds formation, and a lower amount of free bulk water (Gelin et al., 2007). This fast association prevents water evaporation and therefore leads to a reduced WRR. Both of these counteracting effects should be taken into account when performing modifications affecting water loading or release ability.

The structural parameters of BC can be adjusted during the biosynthesis process or by post-synthetic modifications. The addition of certain components to the BC synthetic medium, as well as changes in fermentation conditions, affects the structural properties of the final product in terms of its crystallinity and fiber morphology (Table 1). The ways to increase porosity of harvested BC pellicles were discussed in Chapter 5.1. The dependence of BC water holding and retention abilities on pore size and surface area was investigated by Ul-Islam et al. (2012b). BC was structurally modified through the addition of a single sugar-linked glucuronic acid-based oligosaccharide (SSGO) into the cultural media and via post-synthetic treatment with inorganic Montmorillonite clay. Reductions in porosity and surface area of modified samples resulted in decreased WHC, but improved the WRR characteristic. Other studies also confirmed the relationship between pore size and WHC. BC synthesized in the presence of hydroxypropylmethylcellulose (HPMC) (Huang et al., 2011) showed a more compact structure and reduced porosity, and a reduced WHC value. In contrast, the WHC was improved in BC synthesized in the presence of carboxymethylcellulose (CMC) in the culture media. CMC adhered to the surface of BC fibrils, forming a network with broader ribbons (Chen et al., 2009); Loose fibril arrangement resulted in an increased pore size distribution (Grande et al., 2009a), which corresponded to the greater WHC value (Seifert et al., 2004; Yu et al., 2011). BC with improved porosity and therefore improved water holding/release abilities can be obtained by post-synthetic modifications. The use of foaming agents results in a highly porous BC structure able to absorb at least 7 time more water than is absorbed by pure BC (Yin et al., 2012). Therefore, the control of culture conditions and culture media components, as well as the modifications at a post-synthetic stage, allows the preparation of materials with desired water uptake and release properties through the regulation of BC structural characteristics.

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7.2. The influence of secondary components in the BC structure

Generally, the impregnation of a secondary component into BC fiber network results in reduced pore size due to filling of the pores. However, the nature of the secondary component itself may influence the WHC and WRR values. For example, BC/chitosan composites absorbed larger amounts of water than native BC, despite a reduced porosity, due to the presence of highly hydrophilic chitosan. The capability of chitosan to form hydrogen bonds simultaneously with BC fibrils and with water molecules results in better WHC. At the same time, the reduced porosity improves WRR values, which highlights the importance of additive choice when directing the characteristics of BC towards the desired dressing need (Ul-Islam et al., 2012b). Phisalaphong and Jatupaiboon (2008) also detected increasingly improved water absorption capacity of BC/chitosan films with increasing chitosan content. A dependence of WHC on chitosan molar mass was also observed, with a larger WHC associated with increased molar weight of chitosan. BC/chitosan composites had a more compact structure with smaller pore size, as determined by Lin et al. (2013a). No significant difference was noted in WHC and WRR for modified samples when compared to pure BC, but the dressings provided a suitable moisture environment for wounds with low- and mid-range amounts of exudate. Other highly hydrophilic reagents also affected WHC and WRR values. An increase in water uptake ability was observed when alginate was incorporated into the BC structure, despite the formation of a dense nanoporous structure and a decreased pore diameter (Phisalaphong et al., 2008). Chiaoprakobkij et al. (2011) proposed that the improved WHC of BC/alginate films was associated with the disruption of hydrogen bonds between cellulose fibers due to the blending with the other component. A greater swelling ability observed in the dried sponges was assumed to support better exudate adsorption. The alteration in structure resulting in the dense outer layer might also help to avoid dressing dehydration and prevent bacterial invasion. Another reagent favorable for inclusion in wound dressings is aloe vera gel. When introduced to the structure of BC during the biosynthesis process at a gel content of less than 30%, aloe vera gel improved the WHC by about 1.5 fold compared to the non-modified material. Additional improvement was observed for water vapor permeability values (Saibuatong and Phisalaphong, 2010). The properties of BC composites blended with hydrophilic substances are similar for BC/aloe vera gel, BC/alginate, and BC/chitosan, which indicates the efficiency of composite preparation for the production of materials with adjusted WCH and WRR profiles. An excellent skin

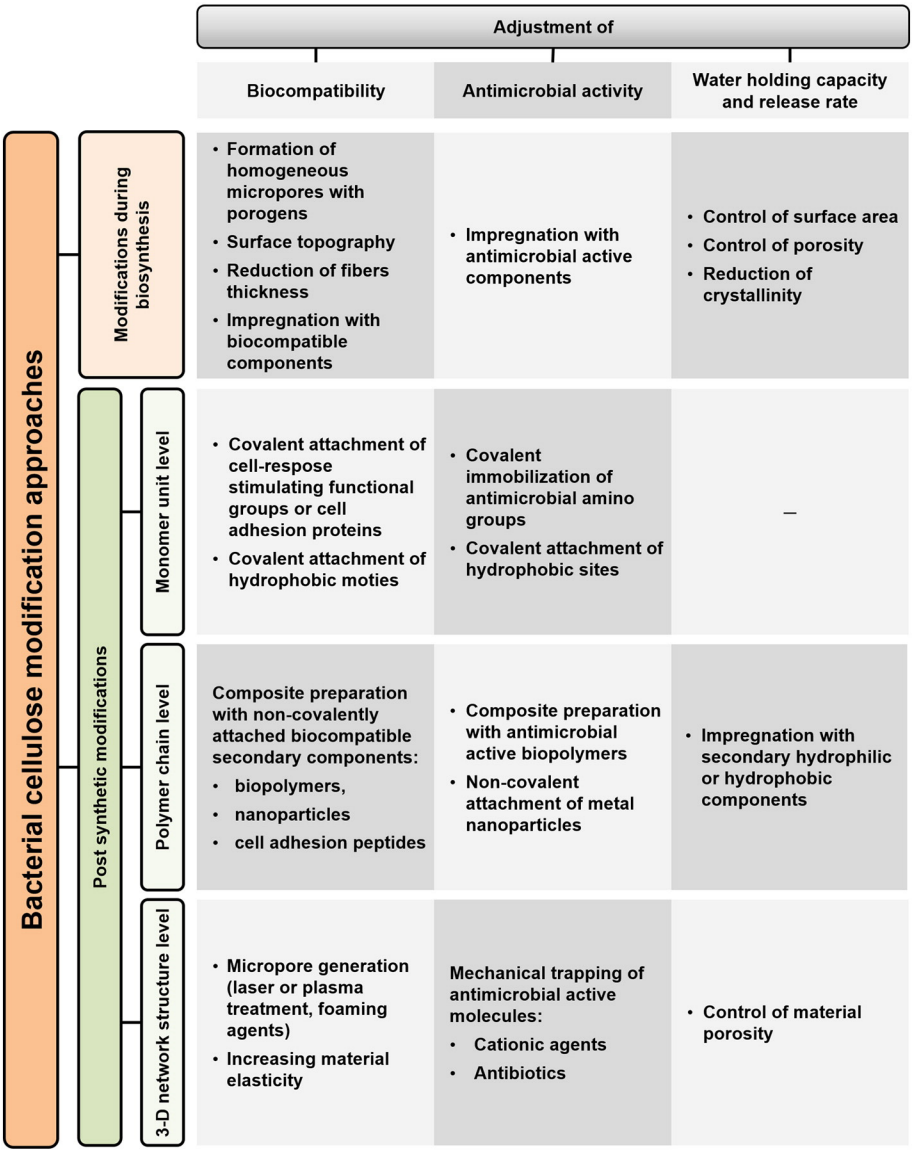

Fig. 6. General overview on possibilities of adjustment bacterial cellulose (BC) characteristics affecting wound recovery mechanisms.

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moisturizing effect was also observed with BC modified with glycerin as a plasticizer. The increased malleability and good biocompatibility suggested the use of this material in dry wound treatment, such as those arising due to psoriasis and atopic dermatitis (Almeida et al., 2014).

Hydrophilic synthetic polymers when impregnated into the BC matrix may significantly alter the liquid absorption characteristics. Thus, BC/poly-AEM composites were shown to increase swelling from 100% for non-modified BC to 6200% for the composite material. The swelling behavior was attributed to the hydrophilic nature of the secondary component together with its ability to prevent the BC structure from collapsing during the drying process (Figueiredo et al., 2015). Great improvement in the swelling ratio of up to 4000–6000% was also observed in BC/acrylic acid hydrogels. In *in vivo* experiments the composites confirmed promotion of burn healing with improved epithelization and fibroblast proliferation which represents it as a promising material for burn dressing production (Mohamad et al., 2014).

8. Conclusions and remarks

The aim of the present work is to provide an overview of the properties of BC as an effective material for wound management and the possible strategies for introducing novel characteristics that would expand the usefulness of BC as a wound treatment. BC itself, without a doubt, has great potential in the fields of biomedicine and biotechnology due to its unique nanoporous 3-D structure of hydrophilic microfibrils. This structural morphology of BC has governed the direction of its utilization as a material for wound dressing production based on its mechanical stability, transparency, biocompatibility, and non-toxicity. BC dressings also protect wounds from bacterial invasion, ensure proper thermal and gaseous exchange, and provide a moist environment for accelerated wound recovery while also adsorbing excess wound exudates. The BC structure guarantees these features, required for an ideal wound dressing, while providing an economical alternative to other moist providing and conventional dressings. The absence of alternative biomaterials that fulfill the challenging requirements to modern wound dressings as well as BC does ensures that bacterial celluloses will be intensively used as a primary material in wound care also in future.

Overall, specific characteristics of BC can be manipulated in a desired direction by structural changes, chemical reactions, or inclusion of secondary components into the BC structure. The modifications can be carried out during the biosynthesis process or by post-synthetic treatment. The intention of this review was to highlight the pathways that can be used to modify the features essential for subsequent utilization of BC as a wound dressing material. This review focused on post production modifications and strategies for improving biocompatibility, antimicrobial activity, and water holding capacity of BC to make it the ideal wound dressing. The general overview on the pathways described in details in corresponding chapters is summarized in the Fig. 6.

Numerous approaches can be taken to influence BC structure to improve its biocompatibility towards desired cell lines, such as introducing functional groups and charges to the BC surface or impregnation with cell adhesion biomolecules or natural or synthetic polymers. Improved biocompatibility may define the primary direction of BC utilization—as a wound dressing material, as a scaffold for tissue engineering, or as a substitute for damaged skin, cartilage, or blood vessels.

Antimicrobial activity is another essential feature for dressings utilized in treatment of chronic wounds, ulcers, and burns. This characteristic can be introduced to BC, again by modifications that alter its structure. Antimicrobial functionalities can be introduced by chemical modification, as well as by impregnation of different antimicrobial agents, such as biopolymers, antimicrobial peptides, cationic antiseptics, antibiotics, or inorganic nanocompounds with strong bactericidal properties. Another important concept of antimicrobial action, the removal of pathogenic organisms from wound media rather than their destruction, is another increasingly attractive possibility for BC treatment of colonized wounds.

The water holding capacity of BC can also be improved via introduction of secondary hydrophilic components or by changing BC structural characteristics, such as increasing the pore size and surface area. Provision of a moist environment by a BC wound dressing can accelerate the healing process and reduce pain.

BC is therefore a unique functional material that has already shown great potential for biomedical applications in its native state, and will certainly continue to do so. Moreover, its potential for modifications and composite formation confirms its favorable standing in modern wound care. In such derivatives, it is a potential basis of many novel materials with admirably optimized properties that can significantly accelerate wound recovery and modernize – or even revolutionize – wound treatment in general. Despite the large number of proposed modification approaches, commercialization of the respective products is far from being realized due to missing confirmation of biocompatibility and biophysiological innocence. In addition, the advantage of using modified material over the non-modified one has to be critically evaluated also with regard to large-scale production: both technological issues and expenses will be critical issues.

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References

Agarwal, A., McAnulty, J.F., Schurr, M.J., Murphy, C.J., Abbott, N.L., 2011. Polymeric materials for chronic wound and burn dressings. In: Farrar, D. (Ed.), Advanced Wound Repair Therapies. Woodhead Publishing, pp. 186–208.

Ahrem, H., Pretzel, D., Endres, M., Conrad, D., Courseau, J., Muller, H., et al., 2014. Laser-structured bacterial nanocellulose hydrogels support ingrowth and differentiation of chondrocytes and show potential as cartilage implants. Acta Biomater. 10, 1341–1353.

Akduman, B., Uygun, M., Coban, E.P., Uygun, D.A., Biyik, H., Akgol, S., 2013. Reversible immobilization of urease by using bacterial cellulose nanofibers. Appl. Biochem. Biotechnol. 171, 2285–2294.

Alavi, A., Sibbald, R.G., Mayer, D., Goodman, L., Botros, M., Armstrong, D.G., et al., 2014. Diabetic foot ulcers: part II. Management. J. Am. Acad. Dermatol. 70, 21 (e1-4).

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P., 2002. Molecular Biology of the Cell. 4th ed. Garland Science, New York.

Almeida, I.F., Pereira, T., Silva, N.H., Gomes, F.P., Silvestre, A.J., Freire, C.S., et al., 2014. Bacterial cellulose membranes as drug delivery systems: an in vivo skin compatibility study. Eur. J. Pharm. Biopharm. 86, 332–336.

Andersson, J., Stenhamre, H., Backdahl, H., Gatenholm, P., 2010. Behavior of human chondrocytes in engineered porous bacterial cellulose scaffolds. J. Biomed. Mater. Res. A 94, 1124–1132.

Andrade, F.K., Costa, R., Domingues, L., Soares, R., Gama, M., 2010a. Improving bacterial cellulose for blood vessel replacement: functionalization with a chimeric protein containing a cellulose-binding module and an adhesion peptide. Acta Biomater. 6, 4034–4041.

Andrade, F.K., Moreira, S.M.G., Domingues, L., Gama, F.M.P., 2010b. Improving the affinity of fibroblasts for bacterial cellulose using carbohydrate-binding modules fused to RGD. J. Biomed. Mater. Res. A 92A, 9–17.

Andrade, F.K., Alexandre, N., Amorim, I., Gartner, F., Mauricio, A.C., Luis, A.L., et al., 2012. Studies on the biocompatibility of bacterial cellulose. J. Bioact. Compat. Polym. 28, 97–112.

Atalla, R.H., Isogai, A., 2010. Celluloses. In: Liu, H.-W., Mander, L. (Eds.), Comprehensive Natural Products II. Elsevier, Oxford, pp. 493–539.

Baah-Dwomoh, A., Rolong, A., Gatenholm, P., Davalos, R., 2015. The feasibility of using irreversible electroporation to introduce pores in bacterial cellulose scaffolds for tissue engineering. Appl. Microbiol. Biotechnol. 99, 4785–4794.

Backdahl, H., Helenius, G., Bodin, A., Nannmark, U., Johansson, B.R., Risberg, B., et al., 2006. Mechanical properties of bacterial cellulose and interactions with smooth muscle cells. Biomaterials 27, 2141–2149.

Backdahl, H., Esguerra, M., Delbro, D., Risberg, B., Gatenholm, P., 2008. Engineering microporosity in bacterial cellulose scaffolds. J. Tissue Eng. Regen. Med. 2, 320–330.

Barud, H.S., de Araújo Júnior, A.M., Santos, D.B., de Assunção, R.M.N., Meireles, C.S., Cerqueira, D.A., et al., 2008. Thermal behavior of cellulose acetate produced from homogeneous acetylation of bacterial cellulose. Thermochim. Acta 471, 61–69.

Barud, H.S., Regiani, T., Marques, R.F.C., Lustri, W.R., Messaddeq, Y., Ribeiro, S.J.L., 2011. Antimicrobial bacterial cellulose-silver nanoparticles composite membranes. J. Nanomater. 2011, 721631.

Please cite this article as: Sulaeva, I., et al., Bacterial cellulose as a material for wound treatment: Properties and modifications. A review, Biotechnol Adv (2015), http://dx.doi.org/10.1016/j.biotechadv.2015.07.009

Basmaji, P., de Olyveira, G.M., dos Santos, M.L., Guastaldi, A.C., 2014. Novel antimicrobial peptides bacterial cellulose obtained by symbioses culture between polyhexanide biguanide (PHMB) and green tea. J. Biomater. Tissue Eng. 4, 59–64.

Basnett, P., Knowles, J.C., Pishbin, F., Smith, C., Keshavarz, T., Boccaccini, A.R., et al., 2012. Novel biodegradable and biocompatible poly(3-hydroxyoctanoate)/bacterial cellulose composites. Adv. Eng. Mater. 14, B330–B343.

Bergstrom, N., Horn, S.D., Smout, R.J., Bender, S.A., Ferguson, M.L., Taler, G., et al., 2005. The national pressure ulcer long-term care study: outcomes of pressure ulcer treatments in long-term care. J. Am. Geriatr. Soc. 53, 1721–1729.

Berndt, S., Wesarg, F., Wiegand, C., Kralisch, D., Müller, F.A., 2013. Antimicrobial porous hybrids consisting of bacterial nanocellulose and silver nanoparticles. Cellulose 20, 771–783.

Bielecki S, Czaja W, Krystynowicz A. A method for the production of bacterial cellulose. Patent WO2005003366 A1; 2005a Jan 13.

Bielecki, S., Krystynowicz, A., Turkiewicz, M., Kalinowska, H., 2005a. Bacterial cellulose. In: Vandamme, E.J., Steinbüchel, A., Baets, S.D. (Eds.), Biopolymers: Volume 5, Polysaccharides I: Polysaccharides from Prokaryotes. Wiley-VCH, pp. 37–90.

Bizot, H., Cathala, B., 2014. A route to uniaxially oriented ribbons of bacterial cellulose nanocrystals based on isomalt spun sacrificial template. Nord. Pulp Pap. Res. J. 29, 15–18.

Bjornberg S, Smith JG. Device for the treatment of vaginal fungal infection. United States patent US 8748689 B2. 2014 Jun 10.

Blake, D.M., Maness, P.-C., Huang, Z., Wolfrum, E.J., Huang, J., Jacoby, W.A., 1999. Application of the photocatalytic chemistry of titanium dioxide to disinfection and the killing of cancer cells. Sep. Purif. Rev. 28, 1–50.

Boateng, J.S., Matthews, K.H., Stevens, H.N., Eccleston, G.M., 2008. Wound healing dressings and drug delivery systems: a review. J. Pharm. Sci. 97, 2892–2923.

Bodin, A., Ahrenstedt, L., Fink, H., Brumer, H., Risberg, B., Gatenholm, P., 2007. Modification of nanocellulose with a xyloglucan–RGD conjugate enhances adhesion and proliferation of endothelial cells: implications for tissue engineering. Biomacromolecules 8, 3697–3704.

Bodin, A., Bharadwaj, S., Wu, S., Gatenholm, P., Atala, A., Zhang, Y., 2010. Tissue-engineered conduit using urine-derived stem cells seeded bacterial cellulose polymer in urinary reconstruction and diversion. Biomaterials 31, 8889–8901.

Bodin, A., Bäckdahl, H., Petersen, N., Gatenholm, P., 2011. Bacterial cellulose as biomaterial. In: Ducheyne, P. (Ed.), Comprehensive Biomaterials. Elsevier, Oxford, pp. 405–410.

Bottan, S., Robotti, F., Jayathissa, P., Hegglin, A., Bahamonde, N., Heredia-Guerrero, J.A., et al., 2015. Surface-structured bacterial cellulose with guided assembly-based biolithography (GAB). ACS Nano 9, 206–219.

Braunwarth, H., Brill, F.H.H., 2014. Antimicrobial efficacy of modern wound dressings: oligodynamic bactericidal versus hydrophobic adsorption effect. Wound Med. 5, 16–20.

Brown, E.E., Laborie, M.-P.G., 2007. Bioengineering bacterial cellulose/poly(ethylene oxide) nanocomposites. Biomacromolecules 8, 3074–3081.

Brumer, H., Zhou, Q., Baumann, M.J., Carlsson, K., Teeri, T.T., 2004. Activation of crystalline cellulose surfaces through the chemoenzymatic modification of xyloglucan. J. Am. Chem. Soc. 126, 5715–5721.

Butcher, M., 2011a. Catch or Kill? How DACC Technology Redefines Antimicrobial Management. MA Healthcare

Butcher, M., 2011b. DACC Antimicrobial Technology: A New Paradigm in Bioburden Management. MA Healthcare.

Butcher, M., 2012. PHMB: an effective antimicrobial in wound bioburden management. Br. J. Nurs. 21 (S16), S8–S21.

Butchosa, N., Brown, C., Larsson, P.T., Berglund, L.A., Bulone, V., Zhou, Q., 2013. Nanocomposites of bacterial cellulose nanofibers and chitin nanocrystals: fabrication, characterization and bactericidal activity. Green Chem. 15, 3404.

Buxboim, A., Discher, D.E., 2012. Mechanical interactions between cells and tissues. In: Matyjaszewski, K., Möller, M. (Eds.), Polymer Science: A Comprehensive Reference. Elsevier, Amsterdam, pp. 201–209.

Cademartiri, R., Anany, H., Gross, I., Bhayani, R., Griffiths, M., Brook, M.A., 2010. Immobilization of bacteriophages on modified silica particles. Biomaterials 31, 1904–1910.

Cai, Z., Kim, J., 2009. Bacterial cellulose/poly(ethylene glycol) composite: characterization and first evaluation of biocompatibility. Cellulose 17, 83–91.

Calum, H., Moser, C., Jensen, P.O., Christophersen, L., Maling, D.S., van Gennip, M., et al., 2009. Thermal injury induces impaired function in polymorphonuclear neutrophil granulocytes and reduced control of burn wound infection. Clin. Exp. Immunol. 156, 102–110.

Chawla, P.R., Bajaj, I.B., Survase, S.A., Singhal, R.S., 2009. Microbial cellulose: fermentative production and applications. Food Technol. Biotechnol. 47, 107–124.

Chen, S., Huang, Y., 2015. Bacterial cellulose nanofibers decorated with phthalocyanine: preparation, characterization and dye removal performance. Mater. Lett. 142, 235–237.

Chen, S., Zou, Y., Yan, Z., Shen, W., Shi, S., Zhang, X., et al., 2009. Carboxymethylated-bacterial cellulose for copper and lead ion removal. J. Hazard. Mater. 161, 1355–1359.

Chen, C.-T., Huang, Y., Zhu, C.-L., Nie, Y., Yang, J.-Z., Sun, D.-P., 2014a. Synthesis and characterization of hydroxypropyl cellulose from bacterial cellulose. Chin. J. Polym. Sci. 32, 439–448.

Chen, H., Liu, Y., Jiang, Z., Chen, W., Yu, Y., Hu, Q., 2014b. Cell-scaffold interaction within engineered tissue. Exp. Cell Res. 323, 346–351.

Cheng, K.-C., Catchmark, J.M., Demirci, A., 2009. Effect of different additives on bacterial cellulose production by *Acetobacter xylinum* and analysis of material property. Cellulose 16, 1033–1045.

Chiaoprakobkij, N., Sanchavanakit, N., Subbalekha, K., Pavasant, P., Phisalaphong, M., 2011. Characterization and biocompatibility of bacterial cellulose/alginate composite sponges with human keratinocytes and gingival fibroblasts. Carbohydr. Polym. 85, 548–553.

Chu, P.K., Chen, J.Y., Wang, L.P., Huang, N., 2002. Plasma-surface modification of biomaterials. Mater. Sci. Eng. R. Rep. 36, 143–206.

Ciechańska, D., 2004. Multifunctional bacterial cellulose/chitosan composite materials for medical applications. Fibres Text. East. Eur. 12, 69–72.

Cui, Q., Zheng, Y., Lin, Q., Song, W., Qiao, K., Liu, S., 2014. Selective oxidation of bacterial cellulose by NO_2–HNO_3. RSC Adv. 4, 1630–1639.

Culebras, M., Grande, C.J., Torres, F.G., Troncoso, O.P., Gomez, C.M., Bañó, M.C., 2015. Optimization of cell growth on bacterial cellulose by adsorption of collagen and poly-L-lysine. Int. J. Polym. Mater. 64, 411–415.

Czaja, W., Krystynowicz, A., Bielecki, S., Brown Jr., R.M., 2006a. Microbial cellulose—the natural power to heal wounds. Biomaterials 27, 145–151.

Czaja, W.K., Young, D.J., Kawecki, M., Brown, R.M., 2006b. The future prospects of microbial cellulose in biomedical applications. Biomacromolecules 8, 1–12.

Czaja, W., Krystynowicz, A., Kawecki, M., Wysota, K., Sakiel, S., Wróblewski, P., et al., 2007. Biomedical applications of microbial cellulose in burn wound recovery. In: Brown Jr., R.M., Saxena, I. (Eds.), Cellulose: Molecular and Structural Biology. Springer, Netherlands, pp. 307–321.

Davidson, J.R., 2015. Current concepts in wound management and wound healing products. Vet. Clin. North Am. Small Anim. Pract. 45, 537–564.

Day, R.M., 2011. Functional requirements of wound repair biomaterials. In: Farrar, D. (Ed.), Advanced Wound Repair Therapies. Woodhead Publishing, pp. 155–173.

de Oliveira, C.R., 2012. Bacterial cellulose membranes constitute biocompatible biomaterials for mesenchymal and induced pluripotent stem cell culture and tissue engineering. J. Tissue Sci. Eng. S11, 005.

de Oliveira, R.L., da Silva, Barud H., de Assunção, R.M.N., da Silva, Meireles C., Carvalho, G.O., Filho, G.R., et al., 2011. Synthesis and characterization of microcrystalline cellulose produced from bacterial cellulose. J. Therm. Anal. Calorim. 106, 703–709.

de Olyveira, M.G., Manzine Costa, M.L., Basmaji, P., 2013. Physically modified bacterial cellulose as alternative routes for transdermal drug delivery. J. Biomater. Tissue Eng. 3, 227–232.

de Santa Maria, L.C., Santos, A.L.C., Oliveira, P.C., Barud, H.S., Messaddeq, Y., Ribeiro, S.J.L., 2009. Synthesis and characterization of silver nanoparticles impregnated into bacterial cellulose. Mater. Lett. 63, 797–799.

de Sousa Andrade, L.N., Pescatore, L.A., Chammas, R., 2015. Cell–Matrix Interactions. Reference Module in Biomedical Sciences. Elsevier.

Dellera, E., Bonferoni, M.C., Sandri, G., Rossi, S., Ferrari, F., Del Fante, C., et al., 2014. Development of chitosan oleate ionic micelles loaded with silver sulfadiazine to be associated with platelet lysate for application in wound healing. Eur. J. Pharm. Biopharm. 88, 643–650.

Dong, C., Qian, L.-Y., Zhao, G.-L., He, B.-H., Xiao, H.-N., 2014. Preparation of antimicrobial cellulose fibers by grafting β-cyclodextrin and inclusion with antibiotics. Mater. Lett. 124, 181–183.

dos Santos, M.V., Dominguez, C.T., Schiavon, J.V., Barud, H.S., de Melo, L.S.A., Ribeiro, S.J.L., et al., 2014. Random laser action from flexible biocellulose-based device. J. Appl. Phys. 115, 083108.

Dufresne, A., 2012. Nanocellulose: From Nature to High Performance Tailored Materials. Walter De Gruyter, Berlin.

Eberlein, T., Haemmerle, G., Signer, M., Gruber-Moesenbacher, U., Traber, J., Mittlboeck, M., et al., 2012. Comparison of PHMB-containing dressing and silver dressings in patients with critically colonised or locally infected wounds. J. Wound Care 21, 12–20.

Esa, F., Tasirin, S.M., Rahman, N.A., 2014. Overview of bacterial cellulose production and application. Agric. Agric. Sci. Procedia 2, 113–119.

Esguerra, M., Fink, H., Laschke, M.W., Jeppsson, A., Delbro, D., Gatenholm, P., et al., 2010. Intravital fluorescent microscopic evaluation of bacterial cellulose as scaffold for vascular grafts. J. Biomed. Mater. Res. A 93A, 140–149.

Evans, B.R., O'Neill, H.M., Malyvanh, V.P., Lee, I., Woodward, J., 2003. Palladium-bacterial cellulose membranes for fuel cells. Biosens. Bioelectron. 18, 917–923.

Farah LFX. Process for the preparation of cellulose film, cellulose film produced thereby, artificial skin graft and its use. United States patent US 4912049; 1990 Mar 27.

Favi, P.M., Benson, R.S., Neilsen, N.R., Hammonds, R.L., Bates, C.C., Stephens, C.P., et al., 2013. Cell proliferation, viability, and in vitro differentiation of equine mesenchymal stem cells seeded on bacterial cellulose hydrogel scaffolds. Mater. Sci. Eng. C Mater. Biol. Appl. 33, 1935–1944.

Fernandes, S.C.M., Oliveira, L., Freire, C.S.R., Silvestre, A.J.D., Neto, C.P., Gandini, A., et al., 2009. Novel transparent nanocomposite films based on chitosan and bacterial cellulose. Green Chem. 11, 2023–2029.

Fernandes, S.C., Sadocco, P., Alonso-Varona, A., Palomares, T., Eceiza, A., Silvestre, A.J., et al., 2013. Bioinspired antimicrobial and biocompatible bacterial cellulose membranes obtained by surface functionalization with aminoalkyl groups. ACS Appl. Mater. Interfaces 5, 3290–3297.

Figueiredo, A.G., Figueiredo, A.R., Alonso-Varona, A., Fernandes, S.C., Palomares, T., Rubio-Azpeitia, E., et al., 2013. Biocompatible bacterial cellulose-poly(2-hydroxyethyl methacrylate) nanocomposite films. BioMed. Res. Int. 2013, 698141.

Figueiredo, A.R.P., Figueiredo, A.G.P.R., Silva, N.H.C.S., Barros-Timmons, A., Almeida, A., Silvestre, A.J.D., et al., 2015. Antimicrobial bacterial cellulose nanocomposites prepared by in situ polymerization of 2-aminoethyl methacrylate. Carbohydr. Polym. 123, 443–453.

Flemming, R.G., Murphy, C.J., Abrams, G.A., Goodman, S.L., Nealey, P.F., 1999. Effects of synthetic micro- and nano-structured surfaces on cell behavior. Biomaterials 20, 573–588.

Fonder, M.A., Mamelak, A.J., Lazarus, G.S., Chanmugam, A., 2007. Occlusive wound dressings in emergency medicine and acute care. Emerg. Med. Clin. North Am. 25, 235–242.

Fonder, M.A., Lazarus, G.S., Cowan, D.A., Aronson-Cook, B., Kohli, A.R., Mamelak, A.J., 2008. Treating the chronic wound: a practical approach to the care of nonhealing wounds and wound care dressings. J. Am. Acad. Dermatol. 58, 185–206.

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Fontana, J.D., de Souza, A.M., Fontana, C.K., Torriani, I.L., Moreschi, J.C., Gallotti, B.J., et al., 1990. Acetobacter cellulose pellicle as a temporary skin substitute. Appl. Biochem. Biotechnol. 24–25, 253–264.

Förch, R., Duque, L., Lotz, A., 2014. 4.18 — antimicrobial bioactive polymer coatings. In: Hashmi, S., Batalha, G.F., Tyne, C.J.V., Yilbas, B. (Eds.), Comprehensive Materials Processing. Elsevier, Oxford, pp. 449–461.

Fu, L., Zhang, Y., Li, C., Wu, Z., Zhuo, Q., Huang, X., et al., 2012. Skin tissue repair materials from bacterial cellulose by a multilayer fermentation method. J. Mater. Chem. 22, 12349–12357.

Fu, L., Zhang, J., Yang, G., 2013. Present status and applications of bacterial cellulose-based materials for skin tissue repair. Carbohydr. Polym. 92, 1432–1442.

Fu, L., Li, Y., Yang, G., 2014. Preparation and characterization of bacterial cellulose/chitosan in situ composites for skin tissue repair and wound healing. Am. Chem. Soc. POLY-477.

Gainza, G., Villullas, S., Pedraz, J.L., Hernandez, R.M., Igartua, M., 2015. Advances in drug delivery systems (DDSs) to release growth factors for wound healing and skin regeneration. Nanomed. Nanotechnol. Biol. Med. 11, 1551–1573.

Gao, C., Wan, Y., Yang, C., Dai, K., Tang, T., Luo, H., et al., 2010. Preparation and characterization of bacterial cellulose sponge with hierarchical pore structure as tissue engineering scaffold. J. Porous. Mater. 18, 139–145.

Gao, C., Wan, Y., Lei, X., Qu, J., Yan, T., Dai, K., 2011. Polylysine coated bacterial cellulose nanofibers as novel templates for bone-like apatite deposition. Cellulose 18, 1555–1561.

Gea, S., Bilotti, E., Reynolds, C.T., Soykeabkeaw, N., Peijs, T., 2010. Bacterial cellulose–poly(vinyl alcohol) nanocomposites prepared by an in-situ process. Mater. Lett. 64, 901–904.

Gelin, K., Bodin, A., Gatenholm, P., Mihranyan, A., Edwards, K., Strømme, M., 2007. Characterization of water in bacterial cellulose using dielectric spectroscopy and electron microscopy. Polymer 48, 7623–7631.

Geng, J., Yang, D., Zhu, Y., Cao, L., Jiang, Z., Sun, Y., 2011. One-pot biosynthesis of polymer–inorganic nanocomposites. J. Nanopart. Res. 13, 2661–2670.

George, J., Ramana, K.V., Bawa, A.S., Siddaramaiah, 2011. Bacterial cellulose nanocrystals exhibiting high thermal stability and their polymer nanocomposites. Int. J. Biol. Macromol. 48, 50–57.

Geyer, U., Heinze, T., Stein, A., Klemm, D., Marsch, S., Schumann, D., et al., 1994. Formation, derivatization and applications of bacterial cellulose. Int. J. Biol. Macromol. 16, 343–347.

Gilbert, P., Moore, L.E., 2005. Cationic antiseptics: diversity of action under a common epithet. J. Appl. Microbiol. 99, 703–715.

Gonçalves, S., Padrão, J., Rodrigues, I.P., Silva, J.P., Sencadas, V., Lanceros-Mendez, S., et al., 2015. Bacterial cellulose as a support for the growth of retinal pigment epithelium. Biomacromolecules 16, 1341–1351.

Grande, C.J., Torres, F.G., Gomez, C.M., Bano, M.C., 2009a. Nanocomposites of bacterial cellulose/hydroxyapatite for biomedical applications. Acta Biomater. 5, 1605–1615.

Grande, C.J., Torres, F.G., Gomez, C.M., Troncoso, O.P., Canet-Ferrer, J., Martínez-Pastor, J., 2009b. Development of self-assembled bacterial cellulose–starch nanocomposites. Mater. Sci. Eng. C 29, 1098–1104.

Gref, R., Domb, A., Quellec, P., Blunk, T., Müller, R.H., Verbavatz, J.M., et al., 2012. The controlled intravenous delivery of drugs using PEG-coated sterically stabilized nanospheres. Adv. Drug Deliv. Rev. 64, 316–326.

Guo, J., Catchmark, J.M., 2012. Surface area and porosity of acid hydrolyzed cellulose nanowhiskers and cellulose produced by *Gluconacetobacter xylinus*. Carbohydr. Polym. 87, 1026–1037.

Guo, L., Yuan, W., Lu, Z., Li, C.M., 2013. Polymer/nanosilver composite coatings for antibacterial applications. Colloids Surf. A Physicochem. Eng. Asp. 439, 69–83.

Ha, J.H., Shah, N., Ul-Islam, M., Khan, T., Park, J.K., 2011. Bacterial cellulose production from a single sugar α-linked glucuronic acid-based oligosaccharide. Process Biochem. 46, 1717–1723.

Haimer, E., Wendland, M., Schlufter, K., Frankenfeld, K., Miethe, P., Potthast, A., et al., 2010. Loading of bacterial bellulose aerogels with bioactive compounds by antisolvent precipitation with supercritical carbon dioxide. Macromol. Symp. 294, 64–74.

Hedlund, C.S., 2007. Surgery of the integumentary systems. In: Fossum, T.W. (Ed.), Small Animal Surgery, 3 ed. Mosby Elsevier, St. Louis, MO, pp. 702–720.

Heinemann, S., 2015. Chapter 1 — polymer-based matrix composites. In: Makhlouf, A.S.H., Scharnweber, D. (Eds.), Handbook of Nanoceramic and Nanocomposite Coatings and Materials. Butterworth-Heinemann, pp. 3–27.

Heinze, T., Liebert, T., 2012. Celluloses and polyoses/hemicelluloses. In: Matyjaszewski, K., Möller, M. (Eds.), Polymer Science: A Comprehensive Reference. Elsevier, Amsterdam, pp. 83–152.

Helenius, G., Backdahl, H., Bodin, A., Nannmark, U., Gatenholm, P., Risberg, B., 2006. In vivo biocompatibility of bacterial cellulose. J. Biomed. Mater. Res. A 76, 431–438.

Henke, M., Tessmar, J., Göpferich, A., 2012. Biomimetic polymers (for biomedical applications). In: Matyjaszewski, K., Möller, M. (Eds.), Polymer Science: A Comprehensive Reference. Elsevier, Amsterdam, pp. 339–361.

Hersel, U., Dahmen, C., Kessler, H., 2003. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. Biomaterials 24, 4385–4415.

Hettegger, H., Sumerskii, I., Sortino, S., Potthast, A., Rosenau, T., 2015. Silane meets click chemistry: towards the functionalization of wet bacterial cellulose sheets. ChemSusChem 8, 680–687.

Hilpert, K., Hancock, R.E.W., 2007. Use of luminescent bacteria for rapid screening and characterization of short cationic antimicrobial peptides synthesized on cellulose using peptide array technology. Nat Protoc. 2, 1652–1660.

Hilpert, K., Elliott, M., Jenssen, H., Kindrachuk, J., Fjell, C.D., Körner, J., et al., 2009. Screening and characterization of surface-tethered cationic peptides for antimicrobial activity. Chem. Biol. 16, 58–69.

Hjertén, S., Wadström, T., 1990. What types of bonds are responsible for the adhesion of bacteria and viruses to native and artificial surfaces? In: Wadström, T., Eliasson, I., Holder, I., Ljungh, Å. (Eds.), Pathogenesis of Wound and Biomaterial-Associated Infections. Springer, London, pp. 245–253

Hong, S.-I., Rhim, J.-W., 2008. Antimicrobial activity of organically modified nano-clays. J. Nanosci. Nanotechnol. 8, 5818–5824.

Hoon R, Mormino R, Serafica G. Microbial-derived cellulose amorphous hydrogel wound dressing. Patent EP 1438975 B1; 2005 Nov 16.

Hou, A., Zhou, M., Wang, X., 2009. Preparation and characterization of durable antibacterial cellulose biomaterials modified with triazine derivatives. Carbohydr. Polym. 75, 328–332.

Hu, W., Chen, S., Yang, J., Li, Z., Wang, H., 2014. Functionalized bacterial cellulose derivatives and nanocomposites. Carbohydr. Polym. 101, 1043–1060.

Huang, H.-C., Chen, L.-C., Lin, S.-B., Chen, H.-H., 2011. Nano-biomaterials application: in situ modification of bacterial cellulose structure by adding HPMC during fermentation. Carbohydr. Polym. 83, 979–987.

Ifuku, S., Tsuji, M., Morimoto, M., Saimoto, H., Yano, H., 2009. Synthesis of silver nanoparticles templated by TEMPO-mediated oxidized bacterial cellulose nanofibers. Biomacromolecules 10, 2714–2717.

Ito, Y., 2011. Growth factors and protein-modified surfaces and interfaces. In: Ducheyne, P. (Ed.), Comprehensive Biomaterials. Elsevier, Oxford, pp. 247–279.

Jampala, S.N., Sarmadi, M., Somers, E.B., Wong, A.C.L., Denes, F.S., 2008. Plasma-enhanced synthesis of bactericidal quaternary ammonium thin layers on stainless steel and cellulose surfaces. Langmuir 24, 8583–8591.

Jeon, J.-H., Oh, I.-K., Kee, C.-D., Kim, S.-J., 2010. Bacterial cellulose actuator with electrically driven bending deformation in hydrated condition. Sensors Actuators B Chem. 146, 307–313.

Jeong, S.I., Lee, S.E., Yang, H., Jin, Y.-H., Park, C.-S., Park, Y.S., 2010. Toxicologic evaluation of bacterial synthesized cellulose in endothelial cells and animals. Mol. Cell. Toxicol. 6, 370–377.

Jiang, T., James, R., Kumbar, S.G., Laurencin, C.T., 2014. Chitosan as a biomaterial: structure, properties, and applications in tissue engineering and drug delivery. In: Kumbar, S., Laurencin, C., Deng, M. (Eds.), Natural and Synthetic Biomedical Polymers. Elsevier, Oxford, pp. 91–113.

Jing, W., Chunxi, Y., Yizao, W., Honglin, L., Fang, H., Kerong, D., et al., 2011. Laser patterning of bacterial cellulose hydrogel and its modification with gelatin and hydroxyapatite for bone tissue engineering. Soft Mater. 11, 173–180.

Jipa, I.M., Stroescu, M., Stoica-Guzun, A., Dobre, T., Jinga, S., Zaharescu, T., 2012. Effect of gamma irradiation on biopolymer composite films of poly(vinyl alcohol) and bacterial cellulose. Nucl. Instrum. Methods B 278, 82–87.

Kane, R.S., Takayama, S., Ostuni, E., Ingber, D.E., Whitesides, G.M., 1999. Patterning proteins and cells using soft lithography. Biomaterials 20, 2363–2376.

Kanjanamosit, N., Muangnapoh, C., Phisalaphong, M., 2010. Biosynthesis and characterization of bacteria cellulose–alginate film. J. Appl. Polym. Sci. 115, 1581–1588.

Katepetch, C., Rujiravanit, R., Tamura, H., 2013. Formation of nanocrystalline ZnO particles into bacterial cellulose pellicle by ultrasonic-assisted in situ synthesis. Cellulose 20, 1275–1292.

Keenan, T.R., 2000. Gelatin. Kirk-Othmer Encyclopedia of Chemical Technology, 6th ed. John Wiley & Sons, Inc, New York, pp. 311–324.

Keshk, S.M.A.S., 2008. Homogenous reactions of cellulose from different natural sources. Carbohydr. Polym. 74, 942–945.

Keshk, S.M., 2014a. Vitamin C, enhances bacterial cellulose production in *Gluconacetobacter xylinus*. Carbohydr. Polym. 99, 98–100.

Keshk, S.M.A.S., 2014b. Bacterial cellulose production and its industrial applications. J. Bioprocess Biotech. 4, 150.

Khan, S., Ul-Islam, M., Khattak, W.A., Ullah, M.W., Park, J.K., 2015. Bacterial cellulose–titanium dioxide nanocomposites: nanostructural characteristics, antibacterial mechanism, and biocompatibility. Cellulose 22, 565–579.

Khayrullin, A.R., Severin, A.V., Khripunov, A.K., Tkachenko, A.A., Pautov, V.D., 2013. Composites based on *Gluconacetobacter xylinus* bacterial cellulose and calcium phosphates and their dielectric properties. Russ. J. Appl. Chem. 86, 1298–1304.

Kim, J., Seo, Y.B., 2002. Electro-active paper actuators. Smart Mater. Struct. 11, 355–360.

Kim, J., Cai, Z., Chen, Y., 2010a. Biocompatible bacterial cellulose composites for biomedical application. J. Nanotechnol. Eng. Med. 1, 011006.

Kim, J., Cai, Z., Lee, H.S., Choi, G.S., Lee, D.H., Jo, C., 2010b. Preparation and characterization of a bacterial cellulose/chitosan composite for potential biomedical application. J. Polym. Res. 18, 739–744.

Kim, S.-S., Jeon, J.-H., Kee, C.-D., Oh, I.-K., 2013. Electro-active hybrid actuators based on freeze-dried bacterial cellulose and PEDOT:PSS. Smart Mater. Struct. 22, 085026.

Kingkaew, J., Kirdponpattara, S., Sanchavanakit, N., Pavasant, P., Phisalaphong, M., 2014. Effect of molecular weight of chitosan on antimicrobial properties and tissue compatibility of chitosan-impregnated bacterial cellulose films. Biotechnol. Bioprocess Eng. 19, 534–544.

Kirdponpattara, S., Phisalaphong, M., 2013. Bacterial cellulose–alginate composite sponge as a yeast cell carrier for ethanol production. Biochem. Eng. J. 77, 103–109.

Klemm, D., Schumann, D., Udhardt, U., Marsch, S., 2001. Bacterial synthesized cellulose — artificial blood vessels for microsurgery. Prog. Polym. Sci. 26, 1561–1603.

Klemm, D., Kramer, F., Moritz, S., Lindström, T., Ankerfors, M., Gray, D., et al., 2011. Nanocelluloses: a new family of nature-based materials. Angew. Chem. Int. Ed. 50, 5438–5466.

Koh, L.-D., Cheng, Y., Teng, C.-P., Khin, Y.-W., Loh, X.-J., Tee, S.-Y., et al., 2015. Structures, mechanical properties and applications of silk fibroin materials. Prog. Polym. Sci. 46, 86–110.

Kolate, A., Baradia, D., Patil, S., Vhora, I., Kore, G., Misra, A., 2014. PEG — a versatile conjugating ligand for drugs and drug delivery systems. J. Control. Release 192C, 67–81.

Kose, R., Sunagawa, N., Yoshida, M., Tajima, K., 2013. One-step production of nanofibrillated bacterial cellulose (NFBC) from waste glycerol using *Gluconacetobacter intermedius* NEDO-01. Cellulose 20, 2971–2979.

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Krystynowicz, A., Czaja, W., Wiktorowska-Jezierska, A., Goncalves-Miskiewicz, M., Turkiewicz, M., Bielecki, S., 2002. Factors affecting the yield and properties of bacterial cellulose. J. Ind. Microbiol. Biotechnol. 29, 189–195.
Kukharenko, O., Bardeau, J.-F., Zaets, I., Ovcharenko, L., Tarasyuk, O., Porhyn, S., et al., 2014. Promising low cost antimicrobial composite material based on bacterial cellulose and polyhexamethylene guanidine hydrochloride. Eur. Polym. J. 60, 247–254.
Kurniawan, H., Lai, J.-T., Wang, M.-J., 2012. Biofunctionalized bacterial cellulose membranes by cold plasmas. Cellulose 19, 1975–1988.
Kwak, M.H., Kim, J.E., Go, J., Koh, E.K., Song, S.H., Son, H.J., et al., 2015. Bacterial cellulose membrane produced by Acetobacter sp. A10 for burn wound dressing applications. Carbohydr. Polym. 122, 387–398.
Lacin, N.T., 2014. Development of biodegradable antibacterial cellulose based hydrogel membranes for wound healing. Int. J. Biol. Macromol. 67, 22–27.
Lagana, G., Anderson, E.H., 2010. Moisture dressings: the new standard in wound care. J. Nurs. Pract. 6, 366–370.
Lai, C., Sheng, L., Liao, S., Xi, T., Zhang, Z., 2013. Surface characterization of TEMPO-oxidized bacterial cellulose. Surf. Interface Anal. 45, 1673–1679.
Lai, C., Zhang, S., Chen, X., Sheng, L., 2014. Nanocomposite films based on TEMPO-mediated oxidized bacterial cellulose and chitosan. Cellulose 21, 2757–2772.
Lee, J.C., Kandula, S., Sherber, N.S., 2009. Beyond wet-to-dry: a rational approach to treating chronic wounds. Eplasty 9, e14.
Legnani, C., Vilani, C., Calil, V.L., Barud, H.S., Quirino, W.G., Achete, C.A., et al., 2008. Bacterial cellulose membrane as flexible substrate for organic light emitting devices. Thin Solid Films 517, 1016–1020.
Li, Y., Wang, S., Huang, R., Huang, Z., Hu, B., Zheng, W., et al., 2015. Evaluation of the effect of the structure of bacterial cellulose on full thickness skin wound repair on a microfluidic chip. Biomacromolecules 16, 780–789.
Lima, GdM, Sierakowski, M.R., Faria-Tischer, P.C.S., Tischer, C.A., 2011. Characterisation of bacterial cellulose partly acetylated by dimethylacetamide/lithium chloride. Mater. Sci. Eng. C 31, 190–197.
Limaye, S.Y., Subramanian, S., Evans, B.R., O'Neill, H.M., 2009 Aug 20. Photoactivated antimicrobial wound dressing and method relating thereto. United States patent US 20090209897 A1.
Lin, Y.K., Chen, K.H., Ou, K.L., Liu, M., 2011. Effects of different extracellular matrices and growth factor immobilization on biodegradability and biocompatibility of macroporous bacterial cellulose. J. Bioact. Compat. Polym. 26, 508–518.
Lin, W.C., Lien, C.C., Yeh, H.J., Yu, C.M., Hsu, S.H., 2013a. Bacterial cellulose and bacterial cellulose–chitosan membranes for wound dressing applications. Carbohydr. Polym. 94, 603–611.
Lin, Y.T., Yu, C.J., Zhu, L., Yin, X.Q., Lao, B.S., Lin, Q., 2013b. Synthesis and characterization of alkyl bacterial cellulose through etherification with alkyl bromide in DMAc/LiCl. Appl. Mech. Mater. 320, 478–482.
Lin, Q., Zheng, Y., Wang, G., Shi, X., Zhang, T., Yu, J., et al., 2015a. Protein adsorption behaviors of carboxymethylated bacterial cellulose membranes. Int. J. Biol. Macromol. 73, 264–269.
Lin, S.-B., Chen, C.-C., Chen, L.-C., Chen, H.-H., 2015b. The bioactive composite film prepared from bacterial cellulose and modified by hydrolyzed gelatin peptide. J. Biomater. Appl. 29, 1428–1438.
Lipsky, B.A., Hoey, C., 2009. Topical antimicrobial therapy for treating chronic wounds. Clin. Infect. Dis. 49, 1541–1549.
Liu, C., Yang, D., Wang, Y., Shi, J., Jiang, Z., 2012. Fabrication of antimicrobial bacterial cellulose–Ag/AgCl nanocomposite using bacteria as versatile biofactory. J. Nanoparticle Res. 14, 1084–1095.
Lopes, T.D., Riegel-Vidotti, I.C., Grein, A., Tischer, C.A., Faria-Tischer, P.C., 2014. Bacterial cellulose and hyaluronic acid hybrid membranes: production and characterization. Int. J. Biol. Macromol. 67, 401–408.
Lu, H., Jiang, X., 2014. Structure and properties of bacterial cellulose produced using a trickling bed reactor. Appl. Biochem. Biotechnol. 172, 3844–3861.
Lu, M., Guan, X.-H., Xu, X.-H., Wei, D.-Z., 2013. Characteristic and mechanism of Cr(VI) adsorption by ammonium sulfamate-bacterial cellulose in aqueous solutions. Chin. Chem. Lett. 24, 253–256.
Luan, J., Wu, J., Zheng, Y., Song, W., Wang, G., Guo, J., et al., 2012. Impregnation of silver sulfadiazine into bacterial cellulose for antimicrobial and biocompatible wound dressing. Biomed. Mater. 7, 065006.
Luo, H., Xiong, G., Yang, Z., Raman, S.R., Si, H., Wan, Y., 2014a. A novel three-dimensional graphene/bacterial cellulose nanocomposite prepared by in situ biosynthesis. RSC Adv. 4, 14369.
Luo, H., Zhang, J., Xiong, G., Wan, Y., 2014b. Evolution of morphology of bacterial cellulose scaffolds during early culture. Carbohydr. Polym. 111, 722–728.
Ma, T., Zhao, Q., Ji, K., Zeng, B., Li, G., 2014. Homogeneous and porous modified bacterial cellulose achieved by in situ modification with low amounts of carboxymethyl cellulose. Cellulose 21, 2637–2646.
Maneerung, T., Tokura, S., Rujiravanit, R., 2008. Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. Carbohydr. Polym. 72, 43–51.
Marins, J.A., Soares, B.G., Barud, H.S., Ribeiro, S.J., 2013. Flexible magnetic membranes based on bacterial cellulose and its evaluation as electromagnetic interference shielding material. Mater. Sci. Eng. C Mater. Biol. Appl. 33, 3994–4001.
Martínez Ávila, H., Schwarz, S., Feldmann, E.-M., Mantas, A., von Bomhard, A., Gatenholm, P., et al., 2014. Biocompatibility evaluation of densified bacterial nanocellulose hydrogel as an implant material for auricular cartilage regeneration. Appl. Microbiol. Biotechnol. 98, 7423–7435.
McKenna, B.A., Kopittke, P.M., Wehr, J.B., Blamey, F.P., Menzies, N.W., 2010. Metal ion effects on hydraulic conductivity of bacterial cellulose–pectin composites used as plant cell wall analogs. Physiol. Plant. 138, 205–214.
Meftahi, A., Khajavi, R., Rashidi, A., Sattari, M., Yazdanshenas, M.E., Torabi, M., 2009. The effects of cotton gauze coating with microbial cellulose. Cellulose 17, 199–204.
Mendes, P.N., Rahal, S.C., Pereira-Junior, O.C., Fabris, V.E., Lenharo, S.L., de Lima-Neto, J.F., et al., 2009. In vivo and in vitro evaluation of an *Acetobacter xylinum* synthesized microbial cellulose membrane intended for guided tissue repair. Acta Vet. Scand. 51, 12.
Millon, L.E., Guhados, G., Wan, W., 2008. Anisotropic polyvinyl alcohol-bacterial cellulose nanocomposite for biomedical applications. J. Biomed. Mater. Res. B Appl. Biomater. 86, 444–452.
Moghimi, S.M., Farhangrazi, Z.S., 2014. Chapter 6 — nanoparticles in medicine: nanoparticle engineering for macrophage targeting and nanoparticles that avoid macrophage recognition. In: Boraschi, D., Duschl, A. (Eds.), Nanoparticles and the Immune System. Academic Press, San Diego, pp. 77–89.
Mogosanu, G.D., Grumezescu, A.M., 2014. Natural and synthetic polymers for wounds and burns dressing. Int. J. Pharm. 463, 127–136.
Mohamad, N., Mohd Amin, M.C.I., Pandey, M., Ahmad, N., Rajab, N.F., 2014. Bacterial cellulose/acrylic acid hydrogel synthesized via electron beam irradiation: accelerated burn wound healing in an animal model. Carbohydr. Polym. 114, 312–320.
Mohite, B.V., Patil, S.V., 2014. Physical, structural, mechanical and thermal characterization of bacterial cellulose by *G. hansenii* NCIM 2529. Carbohydr. Polym. 106, 132–141.
Moreira, S., Silva, N.B., Almeida-Lima, J., Rocha, H.A., Medeiros, S.R., Alves Jr., C., et al., 2009. BC nanofibres: in vitro study of genotoxicity and cell proliferation. Toxicol. Lett. 189, 235–241.
Morgado, P.I., Aguiar-Ricardo, A., Correia, I.J., 2015. Asymmetric membranes as ideal wound dressings: an overview on production methods, structure, properties and performance relationship. J. Membr. Sci. 490, 139–151.
Moritz, S., Wiegand, C., Wesarg, F., Hessler, N., Muller, F.A., Kralisch, D., et al., 2014. Active wound dressings based on bacterial nanocellulose as drug delivery system for octenidine. Int. J. Pharm. 471, 45–55.
Muangman, P., Opasanon, S., Suwanchot, S., Thangthed, O., 2011. Efficiency of microbial cellulose dressing in partial-thickness burn wounds. J. Am. Coll. Certif. Wound Spec. 3, 16–19.
Nge, T.T., Sugiyama, J., Bulone, V., 2010. Bacterial cellulose-based biomimetic composites. In: Elnashar, M. (Ed.), Biopolymers. INTECH Open Access Publisher, pp. 345–368.
Niide, T., Shiraki, H., Oshima, T., Baba, Y., Kamiya, N., Goto, M., 2010. Quaternary ammonium bacterial cellulose for adsorption of proteins. Solvent Extr. Res. Dev. Jpn. 17, 73–81.
Nikaido, H., 1994. Prevention of drug access to bacterial targets: permeability barriers and active efflux. Science 264, 382–388.
Nwe, N., Furuike, T., Tamura, H., 2010. Selection of a biopolymer based on attachment, morphology and proliferation of fibroblast NIH/3T3 cells for the development of a biodegradable tissue regeneration template: alginate, bacterial cellulose and gelatin. Process Biochem. 45, 457–466.
Oliveira Barud, H.G., Barud, HdS, Cavicchioli, M., do Amaral, T.S., Junior, OBdO, Santos, D.M., et al., 2015. Preparation and characterization of a bacterial cellulose/silk fibroin sponge scaffold for tissue regeneration. Carbohydr. Polym. 128, 41–51.
O-Rak, K., Ummartyotin, S., Sain, M., Manuspiya, H., 2013. Covalently grafted carbon nanotube on bacterial cellulose composite for flexible touch screen application. Mater. Lett. 107, 247–250.
Oshima, T., Taguchi, S., Fujiwara, H., Ohe, K., Baba, Y., 2007. Adsorption behaviors of bioactive amines and proteins on phosphorylated bacterial cellulose. J. Ion Exch. 18, 204–207.
Oshima, T., Kondo, K., Ohto, K., Inoue, K., Baba, Y., 2008. Preparation of phosphorylated bacterial cellulose as an adsorbent for metal ions. React. Funct. Polym. 68, 376–383.
Oshima, T., Taguchi, S., Ohe, K., Baba, Y., 2011. Phosphorylated bacterial cellulose for adsorption of proteins. Carbohydr. Polym. 83, 953–958.
Ougiya, H., Hioki, N., Watanabe, K., Morinaga, Y., Yoshinaga, F., Samejima, M., 1998. Relationship between the physical properties and surface area of cellulose derived from adsorbates of various molecular sizes. Biosci. Biotechnol. Biochem. 62, 1880–1884.
Ovington, L.G., 2007. Advances in wound dressings. Clin. Dermatol. 25, 33–38.
Park, M., Chang, H., Jeong, D.H., Hyun, J., 2013. Spatial deformation of nanocellulose hydrogel enhances SERS. Biochip J. 7, 234–241.
Park, Y.B., Lee, C.M., Kafle, K., Park, S., Cosgrove, D.J., Kim, S.H., 2014. Effects of plant cell wall matrix polysaccharides on bacterial cellulose structure studied with vibrational sum frequency generation spectroscopy and X-ray diffraction. Biomacromolecules 15, 2718–2724.
Park, M., Lee, D., Hyun, J., 2015a. Nanocellulose-alginate hydrogel for cell encapsulation. Carbohydr. Polym. 116, 223–228.
Park, S., Park, J., Jo, I., Cho, S.-P., Sung, D., Ryu, S., et al., 2015b. In situ hybridization of carbon nanotubes with bacterial cellulose for three-dimensional hybrid bioscaffolds. Biomaterials 58, 93–102.
Pérez, S., Samain, D., 2010. Structure and engineering of celluloses. Adv. Carbohydr. Chem. Biochem. 64, 25–116.
Pertile, R.A.N., Andrade, F.K., Alves, C., Gama, M., 2010. Surface modification of bacterial cellulose by nitrogen-containing plasma for improved interaction with cells. Carbohydr. Polym. 82, 692–698.
Pertile, R.A., Moreira, S., Costa, R.M., Correia, A., Guardao, L., Gartner, F., et al., 2011. Bacterial cellulose: long-term biocompatibility studies. J. Biomater. Sci. Polym. 23, 1339–1354.
Pertile, R., Moreira, S., Andrade, F., Domingues, L., Gama, M., 2012. Bacterial cellulose modified using recombinant proteins to improve neuronal and mesenchymal cell adhesion. Biotechnol. Prog. 28, 526–532.
Phisalaphong, M., Jatupaiboon, N., 2008. Biosynthesis and characterization of bacteria cellulose–chitosan film. Carbohydr. Polym. 74, 482–488.
Phisalaphong, M., Suwanmajo, T., Tammarate, P., 2008. Synthesis and characterization of bacterial cellulose/alginate blend membranes. J. Appl. Polym. Sci. 107, 3419–3424.
Piatkowski, A., Drummer, N., Andriessen, A., Ulrich, D., Pallua, N., 2011. Randomized controlled single center study comparing a polyhexanide containing bio-cellulose

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dressing with silver sulfadiazine cream in partial-thickness dermal burns. Burns 37, 800–804.
Pierschbacher, M.D., Ruoslahti, E., 1984. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Nature 309, 30–33.
Pinto, R.J.B., Marques, P.A.A.P., Neto, C.P., Trindade, T., Daina, S., Sadocco, P., 2009. Antibacterial activity of nanocomposites of silver and bacterial or vegetable cellulosic fibers. Acta Biomater. 5, 2279–2289.
Pinto, R.J.B., Neves, M.C., Neto, C.P., Trindade, T., 2012. Growth and chemical stability of copper nanostructures on cellulosic fibers. Eur. J. Inorg. Chem. 31, 5043–5049.
Pirich, C., de Freitas, R., Woehl, M., Picheth, G., Petri, D.S., Sierakowski, M., 2015. Bacterial cellulose nanocrystals: impact of the sulfate content on the interaction with xyloglucan. Cellulose 22, 1773–1787.
Pitzer, G.B., Patel, K.G., 2011. Proper care of early wounds to optimize healing and prevent complications. Facial Plast. Surg. Clin. North Am. 19, 491–504.
Pourali, P., Yahyaei, B., Ajoudanifar, H., Taheri, R., Alavi, H., Hoseini, A., 2014. Impregnation of the bacterial cellulose membrane with biologically produced silver nanoparticles. Curr. Microbiol. 69, 785–793.
Putra, A., Kakugo, A., Furukawa, H., Gong, J.P., Osada, Y., Uemura, T., et al., 2007. Production of bacterial cellulose with well oriented fibril on PDMS substrate. Polym. J. (Tokyo Jpn.) 40, 137–142.
Putra, A., Kakugo, A., Furukawa, H., Gong, J.P., Osada, Y., 2008. Tubular bacterial cellulose gel with oriented fibrils on the curved surface. Polymer 49, 1885–1891.
Quinn, K.J., Courtney, J.M., Evans, J.H., Gaylor, J.D.S., Reid, W.H., 1985. Principles of burn dressings. Biomaterials 6, 369–377.
Rai, M., Yadav, A., Gade, A., 2009. Silver nanoparticles as a new generation of antimicrobials. Biotechnol. Adv. 27, 76–83.
Ramani, D., Sastry, T., 2014. Bacterial cellulose-reinforced hydroxyapatite functionalized graphene oxide: a potential osteoinductive composite. Cellulose 21, 3585–3595.
Ring, D.F., Nashed, W., Dow, T., 1986 May 13. Liquid loaded pad for medical applications. United States patent US 4588400 A.
Ring, D.F., Nashed, W., Dow, T., 1987 Apr 7. Pressing, releasing polysaccharide pellicles to rearrange into lamellas. United States patent US4655758 A.
Roach, P., Eglin, D., Rohde, K., Perry, C.C., 2007. Modern biomaterials: a review — bulk properties and implications of surface modifications. J. Mater. Sci. Mater. Med. 18, 1263–1277.
Romano, N.H., Sengupta, D., Chung, C., Heilshorn, S.C., 2011. Protein-engineered biomaterials: nanoscale mimics of the extracellular matrix. BBA Gen. Subj. 1810, 339–349.
Rosenau, T., Renfrew, A.H.M., Adelwöhrer, C., Potthast, A., Kosma, P., 2005. Cellulosics modified with slow-release reagents. Part I. Synthesis of triazine-anchored reagents for slow release of active substances from cellulosic materials. Polymer 46, 1453–1458.
Rosenau, T., Potthast, A., Krainz, K., Hettegger, H., Henniges, U., Yoneda, Y., et al., 2014. Chromophores in cellulosics, XI: isolation and identification of residual chromophores from bacterial cellulose. Cellulose 21, 2271–2283.
Ross, P., Mayer, R., Benziman, M., 1991. Cellulose biosynthesis and function in bacteria. Microbiol. Rev. 55, 35–58.
Rouabhia, M., Asselin, J., Tazi, N., Messaddeq, Y., Levinson, D., Zhang, Z., 2014. Production of biocompatible and antimicrobial bacterial cellulose polymers functionalized by RGDC grafting groups and gentamicin. ACS Appl. Mater. Interfaces 6, 1439–1446.
Roy, D., Knapp, J.S., Guthrie, J.T., Perrier, S., 2007. Antibacterial cellulose fiber via RAFT surface graft polymerization. Biomacromolecules 9, 91–99.
Ruka, D.R., Simon, G.P., Dean, K.M., 2013. In situ modifications to bacterial cellulose with the water insoluble polymer poly-3-hydroxybutyrate. Carbohydr. Polym. 92, 1717–1723.
Sacui, I.A., Nieuwendaal, R.C., Burnett, D.J., Stranick, S.J., Jorfi, M., Weder, C., et al., 2014. Comparison of the properties of cellulose nanocrystals and cellulose nanofibrils isolated from bacteria, tunicate, and wood processed using acid, enzymatic, mechanical, and oxidative methods. ACS Appl. Mater. Interfaces 6, 6127–6138.
Saibuatong, O.-a., Phisalaphong, M., 2010. Novo aloe vera—bacterial cellulose composite film from biosynthesis. Carbohydr. Polym. 79, 455–460.
Sano, M.B., Rojas, A.D., Gatenholm, P., Davalos, R.V., 2010. Electromagnetically controlled biological assembly of aligned bacterial cellulose nanofibers. Ann. Biomed. Eng. 38, 2475–2484.
Santos, S.M., Carbajo, J.M., Quintana, E., Ibarra, D., Gomez, N., Ladero, M., et al., 2015. Characterization of purified bacterial cellulose focused on its use on paper restoration. Carbohydr. Polym. 116, 173–181.
Saska, S., Teixeira, L.N., Tambasco de Oliveira, P., Minarelli Gaspar, A.M., Lima Ribeiro, S.J., Messaddeq, Y., et al., 2012. Bacterial cellulose–collagen nanocomposite for bone tissue engineering. J. Mater. Chem. 22, 22102.
Scherner, M., Reutter, S., Klemm, D., Sterner-Kock, A., Guschlbauer, M., Richter, T., et al., 2014. In vivo application of tissue-engineered blood vessels of bacterial cellulose as small arterial substitutes: proof of concept? J. Surg. Res. 189, 340–347.
Schmitz, M., Eberlein, T., Andriessen, A., 2014. Wound treatment costs comparing a biocellulose dressing with moist wound healing dressings and conventional dressings. Wound Med. 6, 11–14.
Schumann, D.A., Wippermann, J., Klemm, D.O., Kramer, F., Koth, D., Kosmehl, H., et al., 2008. Artificial vascular implants from bacterial cellulose: preliminary results of small arterial substitutes. Cellulose 16, 877–885.
Schütz P, Schultz B. Antimicrobially active wound dressing for catheter fixings. United States patent US 20140194824 A1; 2014 Jul 10.
Seifert, M., Hesse, S., Kabrelian, V., Klemm, D., 2004. Controlling the water content of never dried and reswollen bacterial cellulose by the addition of water-soluble polymers to the culture medium. J. Polym. Sci. A Polym. Chem. 42, 463–470.
Serafica G, Mormino R, Oster GA, Lentz KE, Koehler KP. Microbial cellulose wound dressing for treating chronic wounds. United States patent US 7704523 B2; 2010 Apr 27.
Shah, N., Ul-Islam, M., Khattak, W.A., Park, J.K., 2013. Overview of bacterial cellulose composites: a multipurpose advanced material. Carbohydr. Polym. 98, 1585–1598.
Shao, W., Liu, H., Liu, X., Sun, H., Wang, S., Zhang, R., 2015a. pH-responsive release behavior and anti-bacterial activity of bacterial cellulose-silver nanocomposites. Int. J. Biol. Macromol. 76, 209–217.
Shao, W., Liu, H., Liu, X., Wang, S., Zhang, R., 2015b. Anti-bacterial performances and biocompatibility of bacterial cellulose/graphene oxide composites. RSC Adv. 5, 4795–4803.
Shekaran, A., Garcia, A.J., 2011. Nanoscale engineering of extracellular matrix-mimetic bioadhesive surfaces and implants for tissue engineering. BBA Gen. Subj. 1810, 350–360.
Shezad, O., Khan, S., Khan, T., Park, J.K., 2010. Physicochemical and mechanical characterization of bacterial cellulose produced with an excellent productivity in static conditions using a simple fed-batch cultivation strategy. Carbohydr. Polym. 82, 173–180.
Shi, Q.-S., Feng, J., Li, W.-R., Zhou, G., Chen, A.-M., Ouyang, Y.-S., et al., 2012a. Effect of different conditions on the average degree of polymerization of bacterial cellulose produced by *Gluconacetobacter intermedius* BC-41. Cellul. Chem. Technol. 47, 503–508.
Shi, Q., Li, Y., Sun, J., Zhang, H., Chen, L., Chen, B., et al., 2012b. The osteogenesis of bacterial cellulose scaffold loaded with bone morphogenetic protein-2. Biomaterials 33, 6644–6649.
Shoda, M., Sugano, Y., 2005. Recent advances in bacterial cellulose production. Biotechnol. Bioprocess Eng. 10, 1–8.
Siró, I., Plackett, D., 2010. Microfibrillated cellulose and new nanocomposite materials: a review. Cellulose 17, 459–494.
Skjåk-Bræk, G., Draget, K.I., 2012. Alginates: properties and applications. In: Matyjaszewski, K., Möller, M. (Eds.), Polymer Science: A Comprehensive Reference. Elsevier, Amsterdam, pp. 213–220.
Solway, D.R., Consalter, M., Levinson, D.J., 2010. Microbial cellulose wound dressing in the treatment of skin tears in the frail elderly. Wounds 22, 17–19.
Solway, D.R., Clark, W.A., Levinson, D.J., 2011. A parallel open-label trial to evaluate microbial cellulose wound dressing in the treatment of diabetic foot ulcers. Int. Wound J. 8, 69–73.
Spatz, J.P., Geiger, B., 2007. Molecular engineering of cellular environments: cell adhesion to nano-digital surfaces. In: Yu-Li, W., Dennis, E.D. (Eds.), Methods in Cell Biology. Academic Press, pp. 89–111.
Struszczyk, H., Wrześniewska-Tosik, K., Ciechańska, D., Wesołowska, E., 1995. 3 — effect of the process of biosynthesis on molecular properties of bacterial cellulose. In: Williams, J.F.K.O.P.A. (Ed.), Cellulose and Cellulose Derivatives. Woodhead Publishing, pp. 15–28.
Sun, D., Yang, J., Li, J., Yu, J., Xu, X., Yang, X., 2010. Novel Pd–Cu/bacterial cellulose nanofibers: preparation and excellent performance in catalytic denitrification. Appl. Surf. Sci. 256, 2241–2244.
Sureshkumar, M., Siswanto, D.Y., Lee, C.-K., 2010. Magnetic antimicrobial nanocomposite based on bacterial cellulose and silver nanoparticles. J. Mater. Chem. 20, 6948–6955.
Svensson, A., Nicklasson, E., Harrah, T., Panilaitis, B., Kaplan, D.L., Brittberg, M., et al., 2005. Bacterial cellulose as a potential scaffold for tissue engineering of cartilage. Biomaterials 26, 419–431.
Tang, W., Jia, S., Jia, Y., Yang, H., 2009. The influence of fermentation conditions and post-treatment methods on porosity of bacterial cellulose membrane. World J. Microbiol. Biotechnol. 26, 125–131.
Taokaew, S., Phisalaphong, M., Zhang Newby, B.-m., 2014. In vitro behaviors of rat mesenchymal stem cells on bacterial celluloses with different moduli. Mater. Sci. Eng. C 38, 263–271.
Taokaew, S., Phisalaphong, M., Newby, B-m., 2015. Modification of bacterial cellulose with organosilanes to improve attachment and spreading of human fibroblasts. Cellulose 1–14.
Tejero, R., Anitua, E., Orive, G., 2014. Toward the biomimetic implant surface: biopolymers on titanium-based implants for bone regeneration. Prog. Polym. Sci. 39, 1406–1447.
Thasneem, Y.M., Sharma, C.P., 2013. Chapter 5.1 — in vitro characterization of cell — biomaterials interactions. In: Bandyopadhyay, A., Bose, S. (Eds.), Characterization of Biomaterials. Academic Press, Oxford, pp. 175–205.
Torres, F.G., Commeaux, S., Troncoso, O.P., 2012. Biocompatibility of bacterial cellulose based biomaterials. J. Funct. Biomater. 3, 864–878.
Ul-Islam, M., Khan, T., Khattak, W.A., Park, J.K., 2012a. Bacterial cellulose-MMTs nanoreinforced composite films: novel wound dressing material with antibacterial properties. Cellulose 20, 589–596.
Ul-Islam, M., Khan, T., Park, J.K., 2012b. Water holding and release properties of bacterial cellulose obtained by in situ and ex situ modification. Carbohydr. Polym. 88, 596–603.
Ul-Islam, M., Khattak, W.A., Ullah, M.W., Khan, S., Park, J.K., 2014. Synthesis of regenerated bacterial cellulose–zinc oxide nanocomposite films for biomedical applications. Cellulose 21, 433–447.
Ummartyotin, S., Juntaro, J., Sain, M., Manuspiya, H., 2012. Development of transparent bacterial cellulose nanocomposite film as substrate for flexible organic light emitting diode (OLED) display. Ind. Crop. Prod. 35, 92–97.
Uraki, Y., Nemoto, J., Otsuka, H., Tamai, Y., Sugiyama, J., Kishimoto, T., et al., 2007. Honeycomb-like architecture produced by living bacteria, *Gluconacetobacter xylinus*. Carbohydr. Polym. 69, 1–6.
van der Mei, H.C., Bos, R., Busscher, H.J., 1998. A reference guide to microbial cell surface hydrophobicity based on contact angles. Colloids Surf. B: Biointerfaces 11, 213–221.
Van 'T Hof, W., Veerman, E.C.I., Heimerhorst, E.J., Nieuw Amerongen, A.V., 2001. Antimicrobial peptides: properties and applicability. Biol. Chem. 382, 597–619.
Vincent, L., Engler, A.J., 2011. 5.504 — effect of substrate modulus on cell function and differentiation. In: Ducheyne, P. (Ed.), Comprehensive Biomaterials. Elsevier, Oxford, pp. 51–63.

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von Wilmowsky, C., Bauer, S., Lutz, R., Meisel, M., Neukam, F.W., Toyoshima, T., et al., 2009. In vivo evaluation of anodic TiO_2 nanotubes: an experimental study in the pig. J. Biomed. Mater. Res. B Appl. Biomater. 89B, 165–171.

Waldeck, H.M., Kao, W.J., 2011. 2.207 — extracellular matrix: inspired biomaterials. In: Ducheyne, P. (Ed.), Comprehensive Biomaterials. Elsevier, Oxford, pp. 113–126.

Wan, W., Guhados, G., 2009 Sep 3. Nanosilver coated bacterial cellulose. United States patent US 20090220560 A1.

Wan, Y.Z., Huang, Y., Yuan, C.D., Raman, S., Zhu, Y., Jiang, H.J., et al., 2007. Biomimetic synthesis of hydroxyapatite/bacterial cellulose nanocomposites for biomedical applications. Mater. Sci. Eng. C 27, 855–864.

Wan, Y., Chang, P., Yang, Z., Xiong, G., Liu, P., Luo, H., 2015. Constructing a novel three-dimensional scaffold with mesoporous TiO_2 nanotubes for potential bone tissue engineering. J. Mater. Chem. B 3, 5595–5602.

Wang, Y., Lu, L., Zheng, Y., Chen, X., 2006. Improvement in hydrophilicity of PHBV films by plasma treatment. J. Biomed. Mater. Res. A 76, 589–595.

Wang, Z.-L., Jia, Y., Yi, S., Cong, D.-L., Chen, Y.-Y., Jia, S., et al., 2009. Research on characterization and biocompatibility of bacterial cellulose tissue engineering scaffold. Bioinformatics and Biomedical Engineering, 2009 ICBBE 2009 3rd International Conference on 2009, pp. 1–5.

Wang, J., Wan, Y., Huang, Y., 2012a. Immobilisation of heparin on bacterial cellulose–chitosan nano-fibres surfaces via the cross-linking technique. IET Nanobiotechnol. 6 (2), 52–57.

Wang, J., Wan, Y.Z., Luo, H.L., Gao, C., Huang, Y., 2012b. Immobilization of gelatin on bacterial cellulose nanofibers surface via crosslinking technique. Mater. Sci. Eng. C 32, 536–541.

Wang, J., Jia, H., Zhang, J., Ding, L., Huang, Y., Sun, D., et al., 2014a. Bacterial cellulose whisker as a reinforcing filler for carboxylated acrylonitrile–butadiene rubber. J. Mater. Sci. 49, 6093–6101.

Wang, P., Zhao, J., Xuan, R., Wang, Y., Zou, C., Zhang, Z., et al., 2014b. Flexible and monolithic zinc oxide bionanocomposite foams by a bacterial cellulose mediated approach for antibacterial applications. Dalton Trans. 43, 6762–6768.

Wanichapichart, P., Taweepreeda, W., Nawae, S., Choomgan, P., Yasenchak, D., 2012. Chain scission and anti fungal effect of electron beam on cellulose membrane. Radiat. Phys. Chem. 81, 949–953.

Watson, N.F.S., Hodgkin, W., 2005. Wound dressings. Surgery 23, 52–55.

Wei, B., Yang, G., Hong, F., 2011. Preparation and evaluation of a kind of bacterial cellulose dry films with antibacterial properties. Carbohydr. Polym. 84, 533–538.

Wiegand, C., Elsner, P., Hipler, U.-C., Klemm, D., 2006. Protease and ROS activities influenced by a composite of bacterial cellulose and collagen type I in vitro. Cellulose 13, 689–696.

Williams, D.F., 1999. The Williams Dictionary of Biomaterials. Liverpool University Press.

Wu, J., Zheng, Y., Wen, X., Lin, Q., Chen, X., Wu, Z., 2014a. Silver nanoparticle/bacterial cellulose gel membranes for antibacterial wound dressing: investigation in vitro and in vivo. Biomed. Mater. 9, 035005.

Wu, J., Zheng, Y., Yang, Z., Lin, Q., Qiao, K., Chen, X., et al., 2014b. Influence of dialdehyde bacterial cellulose with the nonlinear elasticity and topology structure of ECM on cell adhesion and proliferation. RSC Adv. 4, 3998–4009.

Xaplanteri, M.A., Andreou, A., Dinos, G.P., Kalpaxis, D.L., 2003. Effect of polyamines on the inhibition of peptidyltransferase by antibiotics: revisiting the mechanism of chloramphenicol action. Nucleic Acids Res. 31, 5074–5083.

Xi, T., Chen, Y.M., Zheng, Y., Guo, T., Hou, J., Wan, Y., et al., 2009. In vitro cytotoxicity of bacterial cellulose scaffolds used for tissue-engineered bone. J. Bioact. Compat. Polym. 24, 137–145.

Xiong, G., Luo, H., Zhang, J., Jin, J., Wan, Y., 2014. Synthesis of ZnO by chemical bath deposition in the presence of bacterial cellulose. Acta Metall. Sin. (Eng. Lett.) 27, 656–662.

Xu, J., Zhu, L., Bai, Z., Liang, G., Liu, L., Fang, D., et al., 2013. Conductive polypyrrole–bacterial cellulose nanocomposite membranes as flexible supercapacitor electrode. Org. Electron. 14, 3331–3338.

Xu, Y., Qiu, C., Zhang, X., Zhang, W., 2014. Crosslinking chitosan into H_3PO_4/HNO_3–$NANO_2$ oxidized cellulose fabrics as antibacterial-finished material. Carbohydr. Polym. 112, 186–194.

Xu, B., Ju, Y., Cui, Y., Song, G., 2015. Carbon nanotube array inducing osteogenic differentiation of human mesenchymal stem cells. Mater. Sci. Eng. C 51, 182–188.

Yamamoto, H., Horii, F., Hirai, A., 2006. Structural studies of bacterial cellulose through the solid-phase nitration and acetylation by CP/MAS ^{13}C NMR spectroscopy. Cellulose 13, 327–342.

Yang, J., Bei, J., Wang, S., 2002. Enhanced cell affinity of poly (D, L-lactide) by combining plasma treatment with collagen anchorage. Biomaterials 23, 2607–2614.

Yang, G., Xie, J., Hong, F., Cao, Z., Yang, X., 2012. Antimicrobial activity of silver nanoparticle impregnated bacterial cellulose membrane: effect of fermentation carbon sources of bacterial cellulose. Carbohydr. Polym. 87, 839–845.

Yang, J., Tang, W., Liu, X., Chao, C., Liu, J., Sun, D., 2013. Bacterial cellulose-assisted hydrothermal synthesis and catalytic performance of La_2CuO_4 nanofiber for methanol steam reforming. Int. J. Hydrog. Energy 38, 10813–10818.

Yang, J., Lv, X., Chen, S., Li, Z., Feng, C., Wang, H., et al., 2014. In situ fabrication of a microporous bacterial cellulose/potato starch composite scaffold with enhanced cell compatibility. Cellulose 21, 1823–1835.

Yang, G., Wang, C., Hong, F., Yang, X., Cao, Z., 2015. Preparation and characterization of BC/PAM–AgNPs nanocomposites for antibacterial applications. Carbohydr. Polym. 115, 636–642.

Yaseen, M., Pan, F., Zhao, X., Lu, J.R., 2011. Enabling technologies: surface modification to improve biocompatibility. In: Moo-Young, Murray (Ed.), Comprehensive Biotechnology, 2nd ed. Elsevier, pp. 65–81.

Yin, X., Yu, C., Zhang, X., Yang, J., Lin, Q., Wang, J., et al., 2010. Comparison of succinylation methods for bacterial cellulose and adsorption capacities of bacterial cellulose derivatives for Cu^{2+} ion. Polym. Bull. 67, 401–412.

Yin, N., Chen, S., Li, Z., Ouyang, Y., Hu, W., Tang, L., et al., 2012. Porous bacterial cellulose prepared by a facile surfactant-assisted foaming method in azodicarbonamide-NaOH aqueous solution. Mater. Lett. 81, 131–134.

Yin, N., Stilwell, M.D., Santos, T.M.A., Wang, H., Weibel, D.B., 2015. Agarose particle-templated porous bacterial cellulose and its application in cartilage growth in vitro. Acta Biomater. 12, 129–138.

Yoshida, Y., Heux, L., Isogai, A., 2012. Heterogeneous reaction between cellulose and alkyl ketene dimer under solvent-free conditions. Cellulose 19, 1667–1676.

Yu, J.W., Liu, X.L., Liu, C.S., Dong, P., 2011. Biosynthesis of carboxymethylated bacterial cellulose composite for wound dressing. Mater. Sci. Forum 685, 322–326.

Zaborowska, M., Bodin, A., Backdahl, H., Popp, J., Goldstein, A., Gatenholm, P., 2010. Microporous bacterial cellulose as a potential scaffold for bone regeneration. Acta Biomater. 6, 2540–2547.

Zahedmanesh, H., Mackle, J.N., Sellborn, A., Drotz, K., Bodin, A., Gatenholm, P., et al., 2011. Bacterial cellulose as a potential vascular graft: mechanical characterization and constitutive model development. J. Biomed. Mater. Res. B Appl. Biomater. 97, 105–113.

Zang, S., Sun, Z., Liu, K., Wang, G., Zhang, R., Liu, B., et al., 2014. Ordered manufactured bacterial cellulose as biomaterial of tissue engineering. Mater. Lett. 128, 314–318.

Zang, S., Zhang, R., Chen, H., Lu, Y., Zhou, J., Chang, X., et al., 2015. Investigation on artificial blood vessels prepared from bacterial cellulose. Mater. Sci. Eng. C 46, 111–117.

Zasloff, M., 2002. Antimicrobial peptides of multicellular organisms. Nature 415, 389–395.

Zeng, X., Liu, J., Chen, J., Wang, Q., Li, Z., Wang, H., 2011. Screening of the common culture conditions affecting crystallinity of bacterial cellulose. J. Ind. Microbiol. Biotechnol. 38, 1993–1999.

Zeugolis, D.I., Raghunath, M., 2011. 2.215 — collagen: materials analysis and implant uses. In: Ducheyne, P. (Ed.), Comprehensive Biomaterials. Elsevier, Oxford, pp. 261–278.

Zhang, T., Wang, W., Zhang, D., Zhang, X., Ma, Y., Zhou, Y., et al., 2010. Biotemplated synthesis of gold nanoparticle-bacteria cellulose nanofiber nanocomposites and their application in biosensing. Adv. Funct. Mater. 20, 1152–1160.

Zhang, J., Chang, P., Zhang, C., Xiong, G., Luo, H., Zhu, Y., et al., 2015. Immobilization of lecithin on bacterial cellulose nanofibers for improved biological functions. React. Funct. Polym. 91–92, 100–107.

Zhao, J., Hu, L., Gong, N., Tang, Q., Du, L., Chen, L., 2015. The effects of macrophage-stimulating protein on the migration, proliferation, and collagen synthesis of skin fibroblasts in vitro and in vivo. Tissue Eng. A 21, 982–991.

Zheng, W., Chen, S., Zhao, S., Zheng, Y., Wang, H., 2014. Zinc sulfide nanoparticles template by bacterial cellulose and their optical properties. J. Appl. Polym. Sci. 131, 40874.

Zhijiang, C., Guang, Y., 2011. Bacterial cellulose/collagen composite: characterization and first evaluation of cytocompatibility. J. Appl. Polym. Sci. 120, 2938–2944.

Zhijiang, C., Guang, Y., Kim, J., 2011. Biocompatible nanocomposites prepared by impregnating bacterial cellulose nanofibrils into poly(3-hydroxybutyrate). Curr. Appl. Phys. 11, 247–249.

Zhijiang, C., Chengwei, H., Guang, Y., 2012. Poly(3-hydroxubutyrate-co-4-hydroxubutyrate)/bacterial cellulose composite porous scaffold: preparation, characterization and biocompatibility evaluation. Carbohydr. Polym. 87, 1073–1080.

Zhu, H., Jia, S., Wan, T., Jia, Y., Yang, H., Li, J., et al., 2011. Biosynthesis of spherical Fe_3O_4/bacterial cellulose nanocomposites as adsorbents for heavy metal ions. Carbohydr. Polym. 86, 1558–1564.

Zhu, C., Li, F., Zhou, X., Lin, L., Zhang, T., 2014a. Kombucha-synthesized bacterial cellulose: preparation, characterization, and biocompatibility evaluation. J. Biomed. Mater. Res. A 102, 1548–1557.

Zhu, L., Qin, J., Yin, X., Ji, L., Lin, Q., Qin, Z., 2014b. Direct sulfation of bacterial cellulose with a $ClSO_3H$/DMF complex and structure characterization of the sulfates. Polym. Adv. Technol. 25, 168–172.

Zou, H., Wu, S., Shen, J., 2008. Polymer/silica nanocomposites: preparation, characterization, properties, and applications. Chem. Rev. 108, 3893–3957.

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