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A multipurpose natural and renewable polymer in medical applications: Bacterial cellulose

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ABSTRACT

Bacterial cellulose (BC) produced by some bacteria, among them *Gluconacetobacter xylinum*, which secrets an abundant 3D networks fibrils, represents an interesting emerging biocompatible nanomaterial. Since its discovery BC has shown tremendous potential in a wide range of biomedical applications, such as artificial skin, artificial blood vessels and microvessels, wound dressing, among others. BC can be easily manipulated to improve its properties and/or functionalities resulting in several BC based nanocomposites. As example BC/collagen, BC/gelatin, BC/Fibroin, BC/Chitosan, *etc*. Thus, the aim of this review is to discuss about the applicability in biomedicine by demonstrating a variety of forms of this biopolymer highlighting in detail some qualities of bacterial cellulose. Therefore, various biomedical applications ranging from implants and scaffolds, carriers for drug delivery, wound-dressing materials, *etc*. that were reported until date will be presented.

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1. Introduction

Natural biopolymers in a variety of biocompatible materials and devices have been the main focus of intense research in medical field and related areas. Consequently, continual efforts from many scientists surely led to the emergence of novel systems that closely mimic the complex and hierarchical structures inherent to the native tissue. The systems or medical devices include wound dressings, medical implants, drug delivery, vascular grafts and scaffolds for tissue engineering (Reis et al., 2008).

Naturally occurring biopolymers viz. collagen, hyaluronan, gelatin, chitosan and cellulose have being explored in biomedicine because their properties are similar to those of the native tissue (Rajwade, Paknikar, & Kumbhar, 2015; Silva et al., 2010).

Particularly, cellulose is the most abundant natural biopolymer on earth, endowed with unique properties and being an ideal starting point for transforming it into useful materials. Cellulose is also present in a wide variety of living species being harvested mainly obtained from trees and cotton.

The first report regarding the production of cellulose from bacteria sources was done by Brown (Brown, 1886) in 1886. The author investigated the biosynthesis of cellulose by *Acetobacter xylinum* – an acetic acid bacteria, that secrets an abundant 3-D network of cellulose fibrils under aerobic conditions, using glucose as a carbon source. The *Acetobacter xylinum* is the most efficient and investigated producer being reclassified thereafter within the genus *Gluconacetobacter xylinus* as *G. xylinus.* The schematic representation of Fig. 1 illustrates the 3-D network of cellulose fibrils derived from bacteria. In terms of composition, bacterial cellulose (BC) is a polymer structurally similar to plant cellulose, however showing superior physicochemical properties (Ul-Islam, Khan, & Park, 2012). This feature is mainly addressed to the well-arranged 3-D network of fibers with diameter ranging from 3.0 to 3.5 μm, which in turn are assembled by bundles of thinner cellulosic fibers with diameter sizes down to micro- and nanoscale. In addition, compared to plant cellulose, BC fibers are free of lignin and hemicellulose (Barud et al., 2011; Klemm, Heublein, Fink, & Bohn, 2005; Svensson et al., 2005).

Considering the strong interaction between hydroxyl groups, BC fibers express a tendency of self-assemble. An extended network is observed *via* both intramolecular and intermolecular hydrogen bonds (Capadona et al., 2009; Li, Lin, & Davenport, 2011) enabling the production of sheets with high surface area and porosity.

Therefore, BC represents an exciting class of nanomaterial and since its discovery has shown tremendous potential as a useful biopolymer which offers a wide range of applications, especially the biomedical ones, including the use as biomaterial for artificial skin, artificial blood vessels and microvessels, wound dressing of second- or third-degree burn ulcers, and dental implants. Other studies with endothelial, smooth muscle cells and chondrocytes have shown that these cells presented good adhesion to BC (Ul-Islam et al., 2012).

Over the past decade, several BC based materials have been designed for a diversity of biomedical applications. There has been a prodigious increase in the number of scientific publication since 2000 as well as an astonishing growth in the number of citations

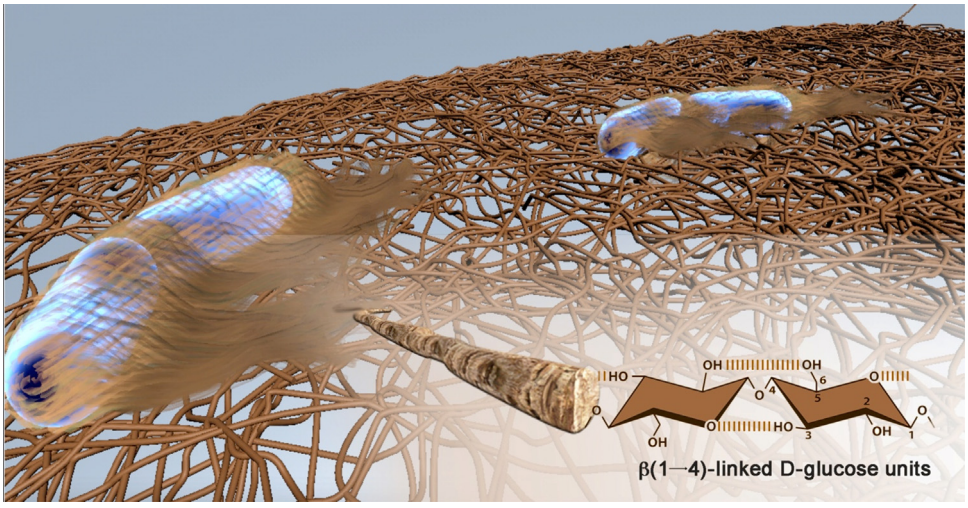

Fig. 1. Representative scheme of the 3-D network secreted by *Acetobacter xylinum* bacteria. In detail, hydroxyl groups of the highlighted sing nanofibril are evidenced.

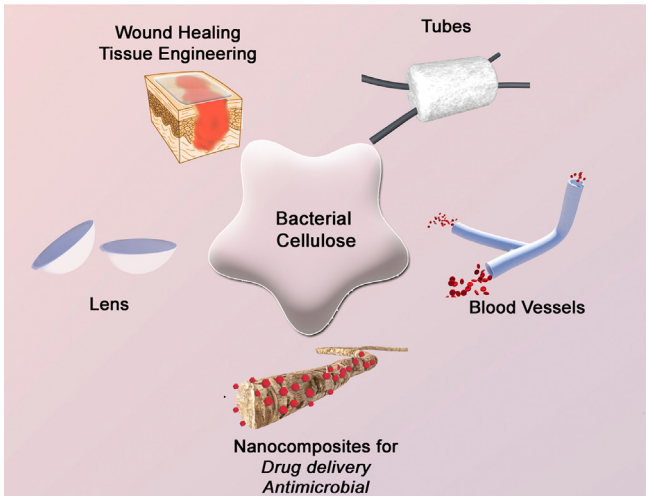

Fig. 2. Some of bacterial cellulose applications in biomedical field.

reporting to BC biomedical materials. In recent years a number of good review articles had highlighted the potential applications of this material (Fu et al., 2012; Fu, Zhang, & Yang, 2013; Jorfi & Foster, 2015; Klemm et al., 2011; Kucinska-Lipka, Gubanska, & Janik, 2015; Rajwade et al., 2015; Shah, Ha, & Park, 2010).

The aim of this review is to discuss about the applicability of BC in biomedicine by demonstrating a variety of forms of this biopolymer either in pristine or as nanocomposites which expands its field of application. Some features of BC will also be highlighted in detail with emphasis on reports that prove its utility in biomedicine. Examples of biomedical applications are schematically shown in Fig. 2.

2. Wound dressings

One of the first proposed and main direct applications of BC membranes in biomedical field is related to wound dressing as Fig. 3 shows. Fontana et al. (1990) were the pioneers in describing the use of BC to replace burned skin. Since then, literature has shown an increasing number of papers related to wound dressing. Cellulose dressings are recommended as a temporary covering for the treatment of wounds, including pressure sores, skin tears, venous stasis, ischemic and diabetic wounds, second-degree burns, skin graft donor sites, traumatic abrasions and lacerations, and biopsy sites by the manufacturers (Kowalska-Ludwicka et al., 2013).

Some BC based wound dressings are in fact available comercially: BioFill®, Bioprocess®, XCell®, and Gengiflex®, for periodontal diseases reconstruction (Farah, 1990).

In terms of ideal wound dressing, the biomembrane BioFill® was one of the first commercial products that fulfils the main prerequisites, including: low cost, good adherence to the wound, water vapor permeability, elasticity, transparency, durability, constitute a physical barrier for bacteria, is hemostatic, easy handling and it can be applicated with minimum exchanges. Additionally, the effectiveness of BioFill® in accelerating the healing process and pain relief has been proven in more than 300 cases (Czaja, Young, Kawecki, & Brown, 2007; Farah, 1990; Wouk et al., 1998).

The analgesic mechanism of action of these wound dressings is not yet fully understood. However, some authors suggest that the healing mechanism involves the capture of ions by means of cellulose hydrogen bonds or the nano BC 3-D network mimics the skin surface creating optimal conditions for healing and regeneration is also proposed (Czaja et al., 2007; Wouk et al., 1998).

It is important to point out that the utilization of BC as a wound dressing clearly shortened the healing time or wound closure over standard care when applied to non-healing lower extremity ulcers, as reported by many researchers (Czaja, Krystynowicz, Bielecki, & Brown, 2006; Czaja et al., 2007; Portal, Clark, & Levinson, 2009).

Wet BC represents a novel class of wound dressing application in the treatment of partial thickness burns as proposed by Czaja et al. (2006, 2007). This type of dressing exhibited outstanding results since wet BC membranes are able to provide a favorable moist environment for a fast wound cleansing, and consequently a faster healing. Likewise, Portal and co-werkers (Portal et al., 2009) applied BC wound dressing (Dermafill™, AMD/Ritmed, Tonawanda, NY) for chronic wounds and found that the mean time for 75% epithelization was reduced from 315 days without the application of BC to 81 days using a BC membrane.

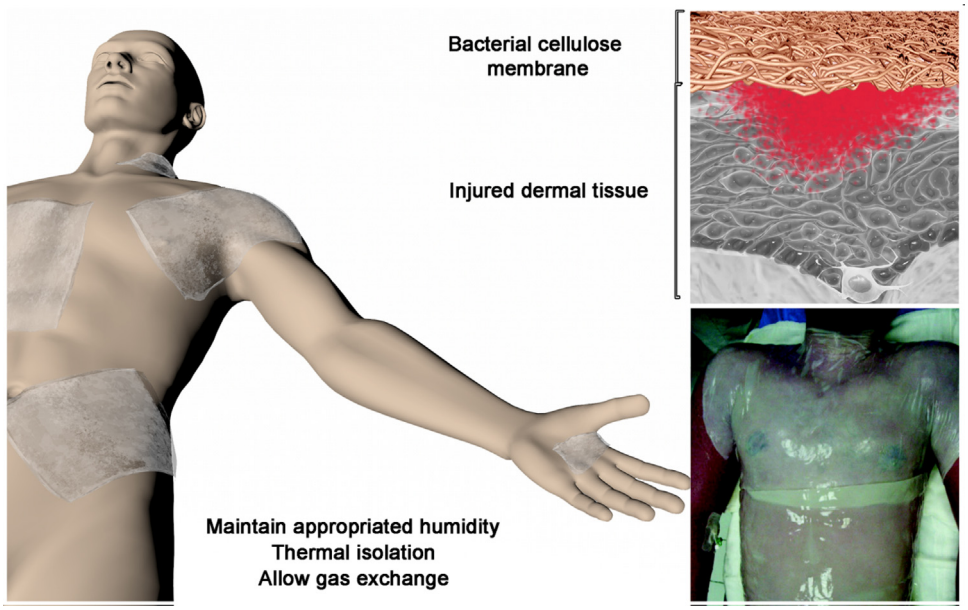

Fig. 3. Representative scheme of BC membrane as a wound dressing. In detail is exemplified the BC network covering the injured.
Source: Adapted from Czaja et al. (2007).

Beyond the direct use above mentioned, improvement and modifications are required to enhance capabilities. BC can be easily manipulated forming nanocomposites with improved properties and/or functionalities. In this way, several BC based nanocomposites have been fabricated. For example, BC/collagen (Albu et al., 2014; Cai & Yang, 2011; Moraes et al., 2016; Saska et al., 2012) and BC/gelatin (Nakayama et al., 2004; Wang, Wan, Luo, Gao, & Huang, 2012) are nanocomposites with improved mechanical properties that have been developed. The mechanical improvements are related to an increase of thermal stability, elastic modulus and tensile strength.

Saibuatong and Phisalaphong (2010) investigated the preparation of BC fibrils and aloe vera nanocomposite films. They obtained bio-polymer film by supplementing 30% (v/v) of aloe gel in the BC culture medium which outcomes BC reinforced fibers with improved properties in terms of mechanical strength, crystallinity, water absorption capacity, and water vapor permeability in comparison with unmodified BC films.

Legeza et al. (2004) produced a BC wound dressing for the treatment of third degree burns that is impregnated with superoxide dismutase (an antioxidant) or poviargol (an antibiotic). Further, BC composite with kaolin (a blood clotting agent) was proved to be a wound healing material as much in a short term as long term (Wanna, Alam, Toivola, & Alam, 2013).

One of the utmost features on the design of wound healing dressing products is the enhancement of water holding capability of the final nanocomposite, in other words, increase the potential to retain water for a long time. Ul-Islam, Khan, Khattak, and Park (2011) found that BC/Chitosan (Ch) composite presented very slow water release. Accordingly, BC-Ch could be applied to the treatment of hard to heal wounds, skin ulcers, bedsores, burns, and wounds that needs frequent dressing changes (Ciechanska, 2004).

Lin, Lien, Yeh, Yu, and Hsu (2013) recently reported the skin wound healing efficacy of BC-Ch composite in experiments assessed with rat models. The authors found that the composite did not produce any toxic effect on animal cells. Moreover, an examination of the tissue regeneration process revealed that wounds treated with BC-Ch composites had epithelialized and regenerated faster than those treated with BC or commercially available dressing materials.

3. Biocompatibility

'Biocompatibility' refers to the ability of a given material to be non-toxic to the biological system, to perform satisfactorily and elicit an appropriate host response upon specific application (Torres, Commeaux, & Troncoso, 2012). Thus, biocompatibility is a result of the complex interactions between an implant and the surrounding tissues. It is a fundamental property, configuring one of the required characteristics to a material be considered a biomaterial.

Due to structural similarities with extracellular matrix components, such as collagen, BC is a highly biocompatible material. Additionally, unlike proteins, the polysaccharide nature of BC makes it less or even non-immunogenic (Petersen & Gatenholm, 2011). There are several *in vitro* and *in vivo* studies in the literature emphasizing the importance of structural characteristic of BC hydrogel and its biocompatibility.

3.1. In vitro *biocompatibility*

Schwann cells were cultured on BC membranes and no significant differences in the morphology and cellular functions were observed on the basis of the results of microscopy (light and scanning electron), cell proliferation assay, flow cytometry and RT-PCR (Zhu, Li, Zhou, Lin, & Zhang, 2014). Native BC allowed the proliferation of L929 cells and human osteoblasts, according to Chen et al. (2009).

Mendes et al. (2009) have assessed the biological response in the presence of a BC membrane after subcutaneous implantation in mice. They performed analysis of histological sections of the BC membrane and the surrounding tissue at 7, 15, 30, 60 and 90 days post-surgery. They found no evidence of foreign body reaction throughout the studied period. Polymorphonuclear cells and lymphocytes were observed at 7, 15 and 30 days post-surgery suggesting a mild inflammatory response. By contrast, at 60 and 90 days post-surgery, no inflammatory cell infiltration was observed.

Human vein endothelial cells exhibited a great proliferation in a BC hydrogel which in turn displayed horizontal growth and occurrence of interesting vertical migration of cells with regards the membrane. Due to the presence of different gradient of oxygen availability as a function of the depth of BC hydrogel, it was found that the cell penetration inside the BC hydrogel was limited up to a certain level of oxygen (Jeong et al., 2010; Recouvreux et al., 2011).

It has been shown that human osteoblasts were able to attach and spread well on larger bacterial cellulose particles obtained in agitated cultures (Hu, Catchmark, & Vogler, 2013).

Kim, Cai, and Chen (2010) prepared BC-gelatin composites to assess the biocompatibility. NIH3T3 fibroblast cells were seeded over pure BC and BC-gelatin composites that were incubated for 48 h. They found that the cells showed good adhesion and proliferation although the biocompatibility was much better in BC-gelatin composites than that of pure BC. Accordingly, the prepared BC-gelatin scaffolds are bioactive, indicating that they can be used for wound dressing and as tissue engineering scaffolds (Kim et al., 2010). Similarly, Wang et al. (2012) have reported the synthesis of a BC/gelatin composite *via* crosslinking by procyanidin. The results showed that the proliferation, infiltration and adhesion of fibroblasts are improved with regard to native BC. Indeed, gelatin, a polypeptide derived from an extracellular matrix, and a denatured form of collagen, exhibits many properties such as good biocompatibility, low immunogenicity, adhesiveness, promotion of cell adhesion and growth and low cost.

Other composites such as BC-poly(ethylene glycol), BC-chitosan and BC-collagen showed better NIH3T3 cell activity as compared to native BC (Cai & Kim 2010; Zhijiang & Guang 2011). Human adipose-derived stem cells proliferated on BC-poly(2-hydroxyethyl methacrylate) to a lower extent in comparison to native BC membranes (Figueiredo et al., 2013).

BC-collagen composites were synthesized for potential tissue engineering applications through an *in situ* synthesis strategy. It should be pointing out that high biodegradability, low antigenicity and cell-binding properties found for BC-collagen composites are distinguished characteristics of a suitable biomaterial with medical purposes (Luo et al., 2008).

As shown throughout this manuscript several studies indicated good biocompatibility of BC, and consequently, it can be inferred that pristine BC membranes would not show genotoxicity and immunoreactivity. Therefore, *in vitro* genotoxicity of BC nanofibers was assessed by single cell gel electrophoresis and the *Salmonella* reversion assays. The reversion assays showed that cellulose nanofibers were non mutagenic or genotoxic and the comet assay indicated no or insignificant DNA damage (Moreira et al., 2009).

Regardless the systemic toxicity, Hagiwara et al., (2010) evaluated the adverse effects of fermentation-derived BC when administered for both sex of F344 rats at dietary for 28 days. No macroscopic changes were observed neither adverse effects were manifested in hematology results. Additionally, no other treatment related changes were apparent observing blood biochemistry

results. Thus, they concluded that BC does not cause any adverse effect when fed to both sex of F344 rats at dietary for 28 days.

3.2. In vivo *biocompatibility*

A detailed systematic evaluation of BC biocompatibility was carried out by Helenius et al. (2006). Upon subcutaneous implantation in Wistar rats, the implants retained their shape without any macroscopic signs of inflammation up to 12 weeks.

Using the improved multilayer fermentation method, BC films were obtained. There were observed low cytotoxicity of the films and good proliferation of human adipose-derived stem cells. Thus, full-thickness skin wounds were made on the backs of BALB/c mice and subsequent histological examinations demonstrated significant fresh tissue regeneration and capillary formation in the wound area of BC groups on day 7 when compared with those commercial dressings and animal skins in other groups. These results indicate the high production efficiency of BC and its high clinical potential is due to the biocompatibility of the films (Fu et al., 2012).

In a similar investigation conducted by Park et al. (2014), BC wound dressing materials were compared with two different commercial dressings, Vaseline gauze and Algisite M, in a rat model. This study showed that BC-dressed animals presented more rapid wound healing on day 14 without any evidence of toxicity when compared to other groups.

Hollow tubes of BC synthesized by rolling method were implanted into the spatium intermusculare region, and data showed that BC did not revealed toxic effects on nerve tissues up to 6 weeks post-implantation (Zhu et al., 2014). Another study on biocompatibility showed that BC/potato starch composite was indeed biocompatible as evidenced by formation of new blood vessels in and around the composite (Yang, Chen, & Wang, 2014).

4. Antimicrobial properties

BC provide a moist environment to a wound resulting in better wound healing. However, in addition to biocompatibility, the second major short coming associated with the medical application of BC is related to its non-bactericidal nature, presenting no antimicrobial activity to prevent wound infection. As a result, several bactericidal elements have been attached to BC to enhance its antimicrobial activities.

It is reported that among the different antimicrobial agents, silver has been the most extensively studied and used since ancient times to fight infections and prevent spoilage (Rai, Yadav, & Gade, 2009). In this way BC-Ag nanocomposites have been prepared through a variety of routes for the same purpose. Remarkably, BC-Ag nanocomposites were found to be effective against many bacterial and fungal species, thereby reducing the chances of wound infection when utilized as dressing materials.

Maneerung, Tokura, and Rujiravanit (2008) obtained a BC-Ag composite by immersing BC in $AgNO_3$ solution, while $NaBH_4$ was applied to reduce the Ag^+ ions adsorbed on the surface of BC nanofibers to produce metallic Ag nanoparticles. The freeze-dried Ag nanoparticle-impregnated BC nanocomposites exhibited strong antimicrobial activity against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive).

Sureshkumar, Siswanto, and Lee (2010) reported an easy method for preparing magnetic BC-Ag nanocomposites. Adding a mixture of Fe^{3+} and Fe^{2+} ions, they first homogenized the 3-D nanofibrous structure of BC and as the pH increased, magnetic NPs were precipitated and attached to the BC surface. A polydopamine layer was then coated onto the magnetic BC nanofibers, which reduced the Ag nanoparticles from $AgNO_3$ solution onto the magnetic BC surface. The magnetization of the prepared BC-Ag nanocomposite was well maintained and impressive antimicrobial activity against the model microbial species have been shown.

Some of us also prepared BC-Ag nanocomposites (Maria et al., 2009) by a simple method loading a large amount of Ag nanoparticles into BC. These composites showed large bactericidal effects, nearly 100% of antibacterial activities against *Escherichia coli* (Maria et al., 2010). Other nanocomposites were obtained by the association of Ag nanoparticles presenting antimicrobial activities (Barud et al., 2008, 2011).

Wu et al. (2014) developed a novel method to synthesize and impregnate Ag nanoparticles onto BC nanofibres (AgNP-BC) to prevent Ag nanoparticles from dropping off 3-D nanofibrous BC structure and thus minimized the toxicity of these nanoparticles. They generated uniform spherical Ag nanoparticles (10–30 nm) and self-assembled on the surface of BC nanofibers forming a stable and evenly distributed hybrid nanostructure. Regardless the slow Ag^+ release, AgNP-BC nanocomposites still exhibited significant antibacterial activities with more than 99% reductions in *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Moreover, AgNP-BC nanocomposites allowed attachment and growth of epidermal cells with no emerged cytotoxicity. The results demonstrated that AgNP-BC nanocomposites could reduce inflammation and promote wound healing.

Still considering metals that exhibit antimicrobial activity, it has been reported that Cu nanoparticles have bactericidal effects comparable to Ag nanoparticles. Thus, Pinto, Neves, Pascoal, and Trindade (2012) reported the antibacterial activity of bionanocomposites made of Cu and BC and plants cellulose as well. The authors reported striking 100% of antibacterial activity against *S. aureus* and *K. pneumoniae*.

Composites of BC membrane impregnated with TiO_2 also have been investigated. The improvement of antibacterial and conducting properties of BC showed that the introduction of TiO_2 substantially promote the use of BC-TiO_2 composites in biomedical applications (Gutierrez, Fernandes, Mondragon, & Tercjak, 2012; Gutierrez, Tercjak, Algar, Retegi, & Mondragon, 2012; Gutierrez, Fernandes, Mondragon, & Tercjak, 2013).

Biomaterials prepared by the addition of different clays and never-dried BC hydrogel have been shown potential use in biomedical applications. Considering the ion exchange properties of montmorrilonite (MMT), researchers extended the work to the preparation of BC composites with modified MMTs (Ul-Islam, Khan, Khattak, & Park, 2013). BC hydrogels impregnated with Ca-MMT, Na-MMT and Cu-MMT were prepared and unveiled significant antibacterial activity (80%) of BC-clay composites.

Propolis has meriting special attention. It is a natural substance with remarkable antifungal, antiviral, antioxidant, anti-inflammatory and antibacterial properties. Barud et al. (2013) produced propolis-BC membranes which were able to incorporate propolis at the surface and interstices. It was evidenced that the polyphenolic compounds determination and the prominent antibacterial activity in the membrane were dose dependent, supporting the possibility of obtaining propolis-BC membranes at the desired concentrations, taking into consideration its application and its skin permanence time. In conclusion, the authors suggested that propolis-BC membrane may favor tissue repair in less time and more effectively in contaminated wounds.

BC-chitosan composites were also found to have both bactericidal and bacteriostatic activities against gram positive and negative bacteria (Ciechanska, 2004). When placed in contact with human fluid containing lysozyme, these composites are degraded resulting in glucosamine and *N*-acetylglucosamine units, which accelerate the wound healing process (Ciechanska, 2004).

Finally, Wei, Yang, and Hong (2011) prepared BC films with benzalkonium chloride, an antimicrobial agent, in order to design a controlled release system to future acute wounds treatment. After

performing *in vitro* antimicrobial tests they concluded that the film was able to reduce the growth and proliferation of gram positive bacteria demonstrating the potential controlled release system designed.

5. Surface modifications of BC for cell adhesion and growth

The surface of native BC generally offers poor cell attachment/adhesion because cellulose is biochemically very inert. It should be pointed out that the interfacial characteristics of biomaterials play a key role in cell adhesion. The surfaces should promote the specific absorption of proteins and subsequent cellular interaction (Angelova, 1999). Several studies have been carried out with the aim of modifying BC surface to optimize the BC-cells interactions. These modifications stand to changes in the physical-chemical properties of BC structures, such as wettability, porosity and surface.

Thus, the modification of surfaces by plasma techniques are becoming common in materials engineering. The most important advantage of this process is the ability to selectively change the surface properties, improving biocompatibility and mimicking the local tissue environment without altering the main attributes. Plasma provides an effective mean to modify surfaces and optimize the biofunctionality (Chu, Chen, Wang, & Huang, 2002). Nitrogen plasma is frequently used to modify metals, polymers and polymeric membranes, aiming the introduction of amino groups in the polymer surface and therefore, changing its polarity, reactivity and wettability (Charpentier, Maguire, & Wan, 2006).

For example, Pertile, Andrade, Alves, and Gama (2010) increased the concentration of the functional amino groups of BC surface with plasma. Thus, adhesion and proliferation studies were performed on nitrogen plasma treated BC membranes by seeding human endothelial cells (HMEC-1) and rat neuroblasts (N1E-115). The results showed a significant increase in the proliferation of cells applied, demonstrating potential applications in tissue engineering. Towards the improvement of cell adhesion, the same groups (Pertile, Moreira, Andrade, Domingues, & Gama, 2012) also investigated the introduction in native BC of small signalling peptides found in the proteins of the extra-cellular matrix (ECMs) such as the integrin-ligand sequences isoleucine-lysine-valine-alanine-valine (IKVAV) mixed to a carbohydrate-binding module (CBM3). These recombinant proteins were adsorbed to BC fibers surface and were able to improve the adhesion of neuronal and mesenchymal cells, but demonstrated no effect on other cell lines tested.

To improve the biocompatibility of BC, basic fibroblast, human epidermal and keratinocyte growth factor were immobilized onto BC surface with different ECMs such as collagen, elastin, and hyaluronan (Lin, Chen, Ou, & Liu, 2011). As a result, human epidermal and collagen-modified BC supported the growth of human skin fibroblast.

The attachment of cells to biomaterials can be improved by utilizing adhesive amino acid sequences, such as Arg-Gly-Asp (RGD), found in several ECMs proteins. Concerning this information, Andrade, Moreira, Domingues, and Gama (2010) conducted a study seeding fibroblasts in RGD protein-modified BC and native BC membranes. Better spreading and uniform distribution of fibroblasts were obtained with the surface modification whereas cell clusters were obtained on native BC membranes. Andrade et al. (2011, 2012), also investigated the biocompatibility of small-diameter BC and peptide (Arg-Gly-Asp)-modified BC membranes subcutaneously implanted in sheep for 1–32 weeks. Peptide-modified BC membranes were mildly irritating to the tissue, with no significant differences in relation to the inflammation degree when compared with expanded polytetrafluoroethylene (ePTFE) thereby used as a negative sample control.

6. Drug delivery

Successful drug delivery systems are influenced by multiple factors and one of which is the appropriate identification of materials for research and engineering of new drug delivery systems. BC is one such biopolymer that fulfils the criteria for consideration as a drug delivery material.

In recent years, several drug-delivery systems based on nanocellulose material for various pharmaceutical applications have been proposed. Inumerous approaches for the preparation of BC-based nanocomposites by incorporating different guest substrates including small molecules, inorganic nanoparticles and polymers on the surfaces of BC nanofibers are exemplified.

The delivery of the antibiotic tetracycline encapsulated on BC matrix was described by Stoica-Guzun, Stroescu, Tache, Zaharescu, and Grosu (2007). They compared irradiated (doses of 5 or 15 kGy) to non-irradiated BC membranes in an *in vitro* study and found that electron beam irradiated over BC-tetracycline system resulted into faster drug release rate.

Lately, researchers applied BC membranes as systems for topical release of lidocaine and ibuprofen (Trovatti et al., 2011, 2012). An *in vitro* drug release study using a phosphate buffer solution (pH 7.4) at 32 °C showed a burst release profile in which more than 90% of the total drug was released in the first 20 min. The therapeutic applicability of different lidocaine delivery systems (BC membrane, a gel, and an aqueous solution) was evaluated *in vitro* with human epidermis. It was found that the permeation rate of lidocaine related to the BC membranes was significantly lower than those obtained with the other two conventional delivery systems (gels and aqueous solutions).

In another study, Müller et al. (2013) investigated BC as potential drug-delivery system for proteins by investigating serum albumin as a model. They found that the freeze-dried BC samples showed a lower uptake of protein than the pristine BC and the biological stability of albumin was maintained during materials processing.

Other models of studies were also described in literature investigating possible effective delivery systems based on BC. Model tablets of Paracetamol (Mohd Amin, Abadi, Ahmad, Katas, & Jamal, 2012) were film coated with BC, using a spray coating technique, and *in vitro* drug release studies of these tablets were investigated. Physicochemical, morphological and thermal properties of BC films were studied. It was found that BC exhibited excellent ability to form soft, flexible and foldable films without the addition of any plasticizer, allowing the delivery of paracetamol, as *in vitro* assays indicates.

BC-caffeine membranes (Silva et al., 2014) were prepared by a simple approach and the permeation of caffeine through human epidermis, from BC or from conventional formulation systems (solution and gel), was compared *in vitro* to assess their therapeutic applicability. Diffusion studies with Franz cells showed that these materials are promising biosystems for topical delivery of caffeine, showing reproducibility and an extended and predictable caffeine release over time, leading to their potential use for cellulite attenuation.

Bacterial cellulose (BC) membranes are used as the carrier for berberine hydrochloride and berberine sulphate to produce a new controlled release system (Huang et al., 2013). Release studies and transdermal experiments were carried out *in vitro*. Carrier BC can significantly extend the drug release time, in contrast to existing commercial carriers that were compared in the study. Freeze-dried BC membranes 10 mm thick were optimized for drug delivery. ^{1}H high-resolution magic angle spinning nuclear magnetic resonance (^{1}diffusion-ordered spectroscopy, DOSY) analysis showed that there was an interaction between the drugs and BC. Despite the structure of BC, the media and the solubility of the drug that can influence the sustained-release behavior, the results indicate

that BC can be a useful material for drug delivery significantly prolonging the release time of the drugs, either for oral administration or transdermally.

Other group of researchers prepared BC-glycerin (Almeida et al., 2014) membranes as supports for drug topical delivery which skin irritation potential of BC was evaluated in human subjects. The good skin tolerance found after a single application under occlusion reinforces the putative interest of BC-glycerin membranes. Besides modifying the mechanical properties, the inclusion of glycerin resulted in a skin moisturizing effect which could be clinically relevant for the treatment for skin diseases characterized by dryness, such as psoriasis and atopic dermatitis.

Mono and multilayer materials from PVA and BC incorporating vanillin as natural antimicrobial ingredient were prepared (Stroescu, Stoica-Guzun, & Jipa, 2013). The composite films were characterized by means of SEM and FTIR. The release mechanism of vanillin from composites films was investigated which diffusion coefficients are ranging from 1.69×10^{-12} to 3.84×10^{-12} m^2 s^{-1}. The vanillin release is influenced by films composition and the multilayer films were found to be promising in order to achieve controlled release of vanillin.

7. Scaffolds

In tissue engineering area a 3D cell-culture system is required to provide the geometrical basis and building blocks to provide cell attachment. Scaffolds are novel systems that closely mimic the complex and hierarchical structures inherent to the native tissue being designed to provide the microenvironment that cells need to proliferate, migrate and differentiate. In addition, some biomechanical properties such as stiffness and elasticity that play important roles in controlling terminal differentiation are also improved (Bäckdahl, Esguerra, Delbro, Risberg, & Gatenholm, 2008).

Several materials have been tested as scaffolds to support growth of cells. Nanocellulose produced by *Gluconacetobacter xylinus* is an emerging biomaterial. As mentioned in Section 1, a pure nanocellulose fibril network is synthesized by the bacteria in any desired shape which microarchitecture and porosity that can be designed by controlling the bacteria fermentation process (Bäckdahl et al., 2008; Rambo et al., 2008). It is also important to point out that the bacterial nanocellulose (BNC) network has a very high affinity for water which results in hydrogel-like properties promoting an ideal environment to host cells (Petersen & Gatenholm, 2011).

Therefore, Bäckdahl et al. (2006) also developed BC scaffolds with controlled microporosity by using paraffin wax and starch particles during culture and removing these particles once the cultivation process finished. The BC scaffolds were then seeded with smooth muscle cells for investigating the potential tissue engineered blood vessel application.

Freeze-drying techniques allowed the preparation BC-poly(ethylene glycol) (PEG) scaffold composites by immersing wet BC pellicle in PEG aqueous solution (Cai & Kim, 2010). Strong interactions between BC and PEG were observed with a decrease in the crystallinity and improvement in thermal stability.

Gao et al. (2011) prepared BC sponge scaffolds using emulsion freeze-drying technique in order to obtain high porosity and consequently, high surface area. The prepared sponges also exhibited excellent cell compatibility and fibrous synovium-derived mesenchymal stem cells (MSCs) could proliferate well on and inside the matrix.

Freeze-drying technique was applied by some of us as well (Oliveira Barud et al., 2015) in the preparation of BC/silk fibroin (SF) sponge scaffolds. *In vitro* tests proved non-cytotoxic or genotoxic character of these nanobiocomposites. SEM images revealed a greater number of fibroblast cells (L929 cell line) attached at the BC/SF:50% scaffold surface if compared with the surface of pure BC, suggesting that the presence of fibroin improved cell attachment as is possible to see in Fig. 4. This could be related to the SF amino acid sequence that act as cell receptors facilitating cell adhesion and growth. Consequently, BC/SF:50% scaffolds configured an excellent option in bioengineering, depicting its potential for tissue regeneration and cultivation of cells on nanobiocomposites.

There is an increased interest in developing adipose tissue as an *in vitro* model for adipose biology and metabolic disease. Krontiras, Gatenholm, and Hagg (2015) recently prepared 2D and 3D porous scaffolds of BC and alginate. The 3D scaffolds were engineered by crosslinking homogenized cellulose fibrils using alginate and freeze-drying the mixture to obtain a porous structure. They found that on 2D surfaces, the cells were scarcely distributed and showed limited formation of lipid droplets, whereas cells grown in macroporous 3D scaffolds contained more cells growing in clusters, containing large lipid droplets. Scaffolds with lower alginate relative content retained their pore integrity better. The authors concluded that 3D culturing of adipocytes in BC macroporous scaffolds is a promising method for fabrication of adipose tissue as an *in vitro* model for adipose biology and metabolic disease. They also suggest that BC-alginate can be used as injectable gel which will be enable to deliver adipose or progenitor cells directly in the defects which is aimed to be repaired.

Other studies have confirmed that different cells, such as human embryonic kidney cells (HEK) (Grande, Torres, Gomez, & Bañó, 2009), bone forming osteoblasts (OB) and fibroblasts (Chen et al., 2009), and human smooth muscle cells (SMC) (Petersen & Gatenholm, 2011) *etc.*, can grow in the presence of BC scaffolds.

Hutmacher (2001) has identified several requirements that tissue engineering scaffolds should fulfill. Among them, biodegradability seems to be the most difficult requirement to meet for BC-based biomaterials, as cellulase enzymes capable of performing cellulose hydrolysis are not present in animals. Li, Wan, Li, Liang, and Wang (2009) have reported the enhancement of the biodegradation of BC *in vitro* (in water, phosphate-buffered saline and simulated body fluid) through periodate oxidation. This chemical treatment preserved the original network structure of BC intact enabling to prepare a BC-based scaffold that could degrade in water, phosphate buffered saline (PBS) and the simulated body fluid (SBF).

In terms of scaffolds, Si et al. (2014) and Luo et al. (2014) prepared Graphene oxide–bacterial cellulose (GO/BC) nanocomposite hydrogels with well-dispersed GO in the network of BC. The *in situ* biosynthesis was developed by adding GO suspension into the culture medium of BC. The experimental results indicate that GO nanosheets are uniformly dispersed and well-bound to the BC matrix and that the 3D porous structure of BC is sustained. This is responsible for efficient load transfer between the GO reinforcement and BC matrix. Compared with the pure BC, the tensile strength and Young's modulus of the GO/BC nanocomposite hydrogel containing 0.48 wt% GO are significantly improved by about 38 and 120%, respectively. The GO/BC nanocomposite hydrogels are promising as a new material for tissue engineering scaffolds.

A BC-alginate scaffold composite (N-BCA) was fabricated by sequential steps of freeze-drying and crosslinking with Ca^{2+} (Kirdponpattara, Khamkeaw, Sanchavanakit, Pavasant, & Phisalaphong, 2015). A mechanically stable structure of N-BCA with open and highly interconnected pores in the range of 90–160 μm was constructed. For long-term culture, the scaffold supported attachment, spreading and proliferation of human gingival fibroblast (GF) on the surface. Because of its biocompatibility and open macroporous structure, N-BCA could potentially be used as a scaffold for tissue engineering.

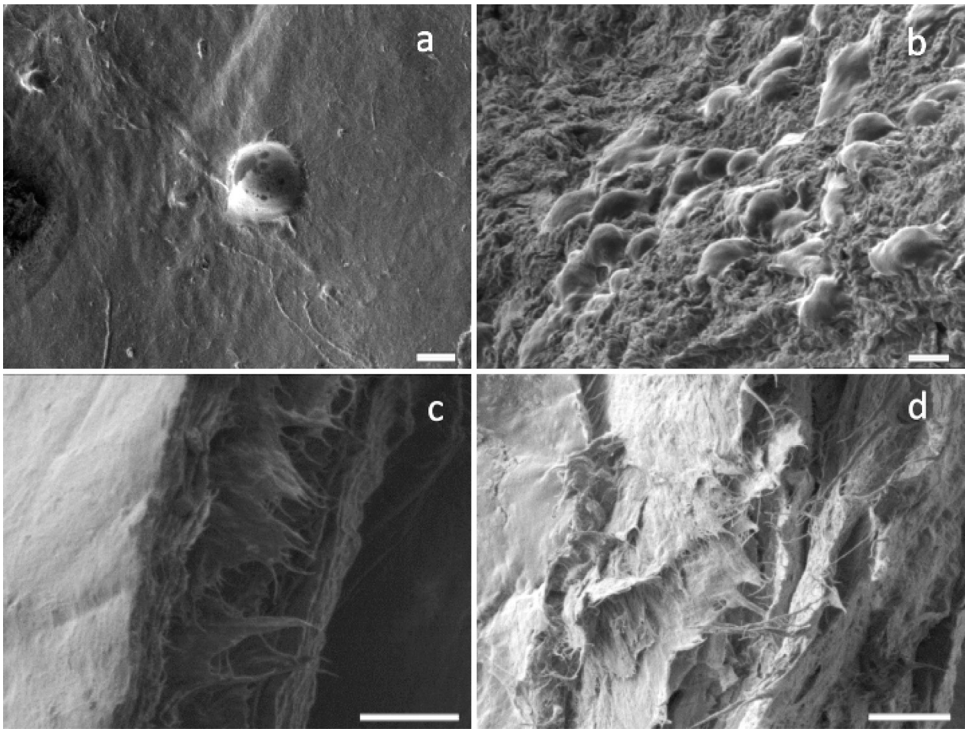

Fig. 4. To test the hypothesis that the addition of silk fibroin to cellulose scaffolds increases cell adhesion (48 h), L-929 cells were seeded in MC and MC/SF scaffolds. SEM images of the cells attached to MC (a) and MC/SF (b) scaffolds surface; cross-section SEM images of MC (c) and MC/SF (d) evidenced that the cells did not migrate into the scaffolds.

Source: Reprinted with permission from Oliveira Barud et al. (2015).

Lecithin is a natural mixture of phospholipids and neutral lipids and the existence of the hydrocarbon groups on the surface was believed to lead to improved blood compatibility (Nakaya & Li, 1999). In an attempt to improve the biological behavior of pristine bacterial cellulose (BC), Zhang et al. (2015) immobilized lecithin (LEC) on the surface of BC nanofibers by solution immersion and subsequent chemical crosslinking with proanthocyanidin (PA). The as-prepared LEC-immobilized BCs (denoted as BC/LECs) were characterized by SEM, FTIR, and XRD, and their dynamic mechanical properties, thermal stability, and hydrophilicity were assessed. It has been found that BC/LECs retain the three-dimensional (3D) porous network structure of pristine BC. The BC/LECs still demonstrate favorable mechanical properties, surface hydrophilicity, and thermal stability. More importantly, preliminary cell studies suggest that the BC/LECs show improved cell behavior over pristine BC.

BC-synthesizing bacteria in medium containing carbon nanotubes (CNTs) coated with an amphiphilic comb-like polymer (APCLP) formed an hybrid scaffold (CNT-BC-Syn) (Park et al., 2015). These scaffolds showed excellent osteoconductivity and osteoinductivity that led to high bone regeneration efficacy whose strategy may open a new avenue for development of 3D biofunctional scaffolds for regenerative medicine.

A stretchable BC nanofiber pellicle was successfully produced by using dissolved oxygen in a conventional cultured medium (Nagashima, Tsuji, & Kondo, 2016). The resulting pellicle could be stretched by up to 1.5 times to provide oriented crystalline nanofibrous films which function was evidenced by direct video imaging of the motion of the bacteria. In conclusion, stressed environment could offer a promising nanofibrous film rich in the cellulose Iα crystalline phase, which opens up the potential of this nanofibrous film for application as a scaffold, reinforcement material, or other structural material.

8. Cardiovascular implants

Processing properties of BC has lead to important applications as artificial blood vessels. Klemm et al. (Klemm, Schumann, Udhardt, & Marsch, 2001; Klemm et al., 2005, 2006; Schumann et al., 2009; Wippermann et al., 2009) have developed prototypes of BC tubes (patented BASYC®-tubes) synthesized in the form of regular tubes with different inner diameter, wall thickness and length, using a patented matrix technique during fermentation. The initial studies showed that the BC tubes have very good surgical handling and can be sterilized in standard ways. In a follow-up *in vivo* study with rats, pigs, and sheep, the BC tubes were successfully used to replace carotid arteries. (Klemm et al., 2001; Schumann et al., 2009; Wippermann et al., 2009).

Similarly, Putra, Kakugo, Furukawa, Gong, and Osada (2008) found that culturing BC in oxygen-permeable silicone tubes with inner diameter <8 mm yields, a tubular BC gel of the desired length, inner diameter, and thickness with uniaxially oriented fibrils were obtained. The tubes presented excellent mechanical properties and holds promise for use as a microvessel or soft tissue material in medical and pharmaceutical applications.

Due to clinical conditions of thrombosis and occlusion, materials that are often used for replacement as vascular grafts are not primarily suitable in small caliber of blood vessels. There is a pursuit for newer non thrombogenic materials with mechanical properties that mimic native vessel which has led to the exploration of BC. The mechanical properties of BC were comparable to porcine carotid artery and were better than expanded polytetrafluorethylene (Bäckdahl et al., 2006). Heparin (Hep) was also hybridized with the BC network to build Hep–BNC nanofibrous scaffolds with anticoagulant properties for potential use in vascular tissue engineering (Wan et al., 2011).

The potential use of BC-based composites for the production of heart valve replacements of cardiovascular tissues was

also reported by Millon and Wan, (2006); Millon, Guhados, and Wan (2008) and Mohammadi (2011). The authors prepared a BC-poly(vinyl alcohol) (PVA) composite that mimics the mechanical behavior of native porcine heart valve leaflets. Furthermore, BC-PVA nanocomposite could exhibit a broad range of mechanical properties aiming at mimicking not only the non-linear mechanical properties displayed by porcine heart valves, but also their anisotropic behavior. This property is related to the ability of the prepared material in withstanding tensile forces depending on the direction of the fibers. This peculiarity emphasizes the importance of this material when used as a vascular graft by controlling material and processing parameters (Millon et al., 2008; Mohammadi, 2011).

9. Cartilage/meniscus implants

Due to the limited regeneration capacity of the cartilage tissue, the repair of cartilage defects configures a challenge and a clinical need. Materials for artificial cartilage are required to be not only tough and resistant but also proof of biodegradation. As BC presents excellent mechanical properties and low biodegradability, chondrocytes were seeded on BC membranes and showed proliferation and collagen type II production, indicating suitability as a bio-mimicking scaffold for cartilage replacement (Svensson et al., 2005).

Bodin, Concaro, Brittberg, and Gatenholm (2007) compared the mechanical properties of a BC gel with traditional collagen meniscal implant material and real pig meniscus. It was found that the Young's modulus of BC gel is similar to the one of pig meniscus, and five times higher than the one of collagen material. The results of promising cell migration and controlled meniscus shape indicated that BC can be an attractive material as meniscus implant.

Another study conducted by Lopes et al. (2011) investigated the friction and wear behaviors of BC pellicles against bovine articular cartilage. Due to the wear mechanism involving high plastic deformation, BC biomaterials possess low friction coefficient values (about 0.05) and preservation of the mating surfaces. This BC biomaterial was reported to be a potential replacement of artificial cartilage for articular joints.

In order to mimic the ultrastructure of the central region of the knee meniscus, Martínez, Brackmann, Enejder, and Gatenholm (2012) fabricated BC devices together with micro-channels ($Ø \sim 500\ \mu m$). Results showed that the micro-channels could facilitate the alignment of cells and collagen fibers, and the parallel orientation of collagen fibers in contrast strengthen the tissues, making it suitable for knee meniscus and tendons replacement.

Articular chondrocytes from young adult patients as well as neonatal articular chondrocytes were seeded with various seeding techniques onto the porous BC scaffolds. Furthermore, DNA analysis implied that the chondrocytes proliferated within the porous BC and with some further development, this novel biomaterial can be a suitable candidate for cartilage regeneration applications according to Andersson, Stenhamre, Bäckdahl, and Gatenholm (2010).

Recently, Ávila et al. (2014) proposed a non-resorbable implant material for auricular cartilage replacement based on BC with 15% of cellulose content, since it matches the mechanical strength and mainly the host tissue response of human auricular cartilage.

In terms of BC composites that presents applications in cartilage tissues, Azuma et al. (2007) concluded that BC-poly(dimethyl acrylamide) double network gel has mechanical properties similar to the mechanical properties of cartilage and that may meet the requirements of artificial cartilage.

10. Bone and connective tissue repair

Bone is a composite material comprising basically an organic phase (collagen and noncollagenous proteins) and an inorganic mineral phase (calcium hydroxyapatite). Nanocellulose and its biocomposites have been proved to be promising materials for the culture of various cells, including osteoblast and chondroblast, indicating that nanocellulose based materials have the potential for bone tissue regeneration and healing.

BC can be a good matrix for obtaining different types of calcium carbonate ($CaCO_3$) crystals with improved biocompatibility. Stoica-Guzun et al. (2012) have used calcium chloride ($CaCl_2$) and sodium carbonate (Na_2CO_3) as starting reactants to promote calcium carbonate deposition on BC membranes.

A membrane composed of BC and hydroxyapatite (Hap) was developed as biomaterial for potential bone regeneration, which delivered prone growth of osteoblast cells, high level of alkaline phosphatase activity, and greater bone nodule formation (Tazi et al., 2012). The better osteoblasts adhesion, proliferate and mineralization from BC-Hap biomaterials were expected to facilitate quick regeneration of bone tissue. Similarly, Grande et al. (2009) produced BC-Hap scaffolds for biomedical applications and obtained excellent results in terms of regeneration of bone and connective tissues.

Researchers also prepared and characterized BC-Hap composites (Hong et al., 2006; Wan et al., 2006). They found that the HAp crystals are partially substituted with carbonate, resembling natural bones. The nanocomposites containing HAp with structural features close to those of biological apatites are attractive for applications as artificial bones and scaffolds for tissue engineering.

Saska et al. (2011) prepared BC-Hap nanocomposites. They evaluated the biological properties and performance of the material with respect to bone regeneration in defects of rat tibiae (Saska et al., 2011). The BC-Hap membranes were effective for bone regeneration and accelerated new bone formation. In addition, reabsorption of the membranes was slow, suggesting that it takes longer to this composite to be completely reabsorbed.

Other authors (Wan et al., 2009; Yin et al., 2011) have induced a negative charge on BC by the adsorption of polyvinylpyrrolidone (PVP) to initiate the nucleation of Hap *via* dynamic simulated body fluid treatment. Shi et al. (2009) introduced an alkaline treatment before the biomimetic mineralization process in order to improve the mineralization efficiency. On the other hand, Zhang et al. (2009) have used a phosphorylation reaction to introduce phosphate groups to the surface of BC and promote the growth of calcium phosphate. Wan et al., (2009) have also shown that phosphorylation effectively triggers Hap formation on BC which allowed BC-Hap composites with a third phase.

Recently, Pigossi et al. (2015) evaluated the potential of BC-Hap composites associated with osteogenic growth peptide (OGP) or pentapeptide OGP (10–14) in bone regeneration in critical-size calvarial defects in mice. OGP is proteolytically cleaved, thus generating the osteogenic growth peptide C-terminal pentapeptide (NH2-YGFGG-OH), named OGP(10–14). OGP (10–14) may be the physiologically active form of OGP because it is this C-terminal pentapeptide that activates the cytoplasmic OGP signalling pathway (Gabarin et al., 2001). Therefore, this suggests that OGP (10–14) is the bioactive form of OGP (Chen et al., 2002; Greenberg et al., 1993). In this study, the BC-Hap, BC-Hap-OGP, and BC-Hap-OGP (10–14) membranes were analyzed at 3, 7, 15, 30, 60, and 90 days. In each period, the specimens were evaluated by micro-computed tomography (μCT), descriptive histology, gene expression of bone biomarkers by qPCR and VEGFR-2 (vascular endothelial growth factor) quantification by ELISA. The researchers found that at 60 and 90 days, a high percentage of bone formation was observed by μCT for BC-Hap and BC-Hap-OGP (10–14) membranes. High expres-

sion of some bone biomarkers, such as Alpl, Spp1, and Tnfrsf11b, was also observed. They concluded that the BC-HA membrane promoted a better bone formation in critical-size mice calvarial defects.

Fan et al. (2013) reported the fabrication of novel bone repair biomaterials with the introduction of goat bone apatite in BC. The produced biomaterial can stimulate bone cells proliferation and promote cell differentiation as demonstrated *in vitro* assays. Noteworthy, Lee, Kim, Lee, and Park (2013) evaluated *in vivo* assays by implanting silk fibroin-BC membranes to successfully promote the complete healing of segmental defects of zygomatic arch of rats.

11. Dental/oral implants

Some of the first applications in dental field were related to the regeneration of periodontal disease through guided tissue regeneration technique (GTR). Novaes and Novaes (1992, 1993) reported adequate GTR results in periodontal defects in humans as well as in GTR for bone formation in association with osseointegrated implants using the commercial BC membrane, Gengiflex®.

Chiaoprakobkij, Sanchavanakit, Subbalekha, Pavasant, and Phisalaphong (2011) developed a composite based on bacterial cellulose/alginate to be used as a temporary dressing of surgical wounds of the oral mucosa. The material features a unique design: the outer layer is thicker to prevent bacterial contamination and dehydration of the wound while the inner layer is porous and intended to drain exudates. Preliminary *in vitro* tests of biocompatibility showed a good performance of the material which enabled the proliferation of keratinocytes and gingival fibroblasts.

BC was also reported as an innovative material for dental root canal treatment in animal experiments. In comparison with conventional paper point materials, BC exhibited greater compatibility and biological characteristics for dental root canal treatment. The absorption rate of BC-based biomaterials was about 10-fold greater than that of paper point materials, and BC-based biomaterials can preserve better tensile strength under wet condition and, in addition, it showed maintenance of physical integrity and only a small foreign body reaction (Yoshino et al., 2013).

Cellulose whiskers may be also obtained from BC. Nanometric scale whiskers (nanowhiskers) can be used in materials reinforcement. Jinga et al. (2014) prepared BC nanowhiskers and used commercial MTA (Mineral Trioxide Aggregates) cement for the preparation of some composites: MTA-E (Mineral Trioxide Aggregates-experimental), MTA-10% biocell and MTA-33% biocell cements. BC was observed to accelerate the hardening processes of MTA cement, decreasing in the same time the relative quantity of calcium hydroxide crystals.

12. Neural implants/dura máter

Nervous tissue reconstruction is a really challenging problem. Self-regeneration may be difficult depending on the extent of the harm. In this context, BC was assessed as a substitute for dura mater in Mongrel dogs. Macroscopic examination of the grafts demonstrated good acceptance and adherence to the bone fragment (Mello, Feltrin, Neto, & Ferraz, 1997). Pertile et al. (2012) reported the application of BC as a scaffold for nerve tissue regeneration, where BC fibers maintained a continuous path that promoted infiltration of cells. The researchers showed that mesenchymal stem cells adhered to BC proliferated and expressed nerve growth factor neurotrophin thus creating a microenvironment that promotes neuronal regeneration.

BC was also reported to be developed as biomaterial for the reconstruction of damaged peripheral nerves *via* cellulosic guidance channels. *In vivo* experiments were conducted on the femoral nerve of Wistar rats for three months. Results evaluation from histological analysis and postoperative observation of motor recovery showed that BC neurotubes can effectively prevent the formation of neuromas, while allowing the accumulation of neurotrophic factors inside, and facilitating the process of nerve regeneration (Kowalska-Ludwicka et al., 2013).

The preparation of nerve conduits for repairing peripheral nerve injuries was explored using BC for it. *In vitro* data indicated that BC is biocompatible with Schwann cells and presented no adverse hematological and histological effects upon *in vivo* implantation in rats (Zhu et al., 2014).

Xu et al. (2014) applied BC to repair dural defects in rabbits. Despite the long-term effect of this new dural material needs to be validated in larger animals, results showed that BC exhibited a decreased inflammatory response compared to traditional materials.

13. Artificial cornea/contact lens

This is another interesting applicability of BC that is also few documented in literature. A Brazilian research group developed and patented (Messaddeq, Ribeiro, & Thomazini, 2008) a special mechanism to conform BC into correct angles and shape to produce contact lenses for cornea regeneration. Wet BC membranes are cut in a round shape and then are compressed with the stick that presents a semi-spherical end onto the base, under a constant 150 °C heat. Thus, there can be obtained contact lens shaped internally by the compression stick and externally by the mold, as shown in Fig. 5.

In an effort to extend this work, Cavicchioli et al. (2015) impregnated ciprofloxacin (CPX) with and without 2-hydroxypropyl-γ-cyclodextrin (γCD) into BC membrane in the shape of a contact lens by using aforementioned method in order to improve their therapeutic potential. Pure and impregnated membranes did not exhibit cytotoxicity, genotoxicity or mutagenicity effects. Otherwise, the BC-CPX membrane was only cytotoxic. They concluded that, except for BC-CPX, the investigated materials are promising for biomedical applications, especially as a contact lens used for regeneration or protection against bacteria.

14. Urinary conduits

According to the American Cancer Society, bladder cancer is the second most common urologic malignancy in the United States, after prostate cancer. The chance of developing bladder cancer is about 1 in 27 for men and 1 in 85 for women. In order to treat malignancies that have invaded the bladder muscle, surgical resection of the tumor, followed by the creation of a continent urinary reservoir using segments of the small or large intestine is often necessary. Urinary diversion after radical cystectomy in patients with bladder cancer normally takes the form of an ileal conduit or neobladder. However, such diversions are associated with a number of complications including increased risk of infection. A plausible alternative is the construction of a neobladder (or bladder tissue) *in vitro* using autologous cells harvested from the patient. Biomaterials can be used as a scaffold for naturally occurring regenerative stem cells to latch onto to regrow the bladder smooth muscle and epithelium. Such engineered tissues show great promise in urologic tissue regeneration, but are faced with a number of challenges.

Thus, Bodin et al. (2010) produced microporous BC scaffolds seeded with human urine-derived stem cells to form a tissue-engineered conduit for use in urinary diversion. The cells were also induced to differentiate into urothelial and smooth muscle cells.

The effects of urethral reconstruction with a three-dimensional (3D) porous BC scaffold prepared by gelatin sponge interfering in the BC fermentation process and seeded with lingual keratinocytes

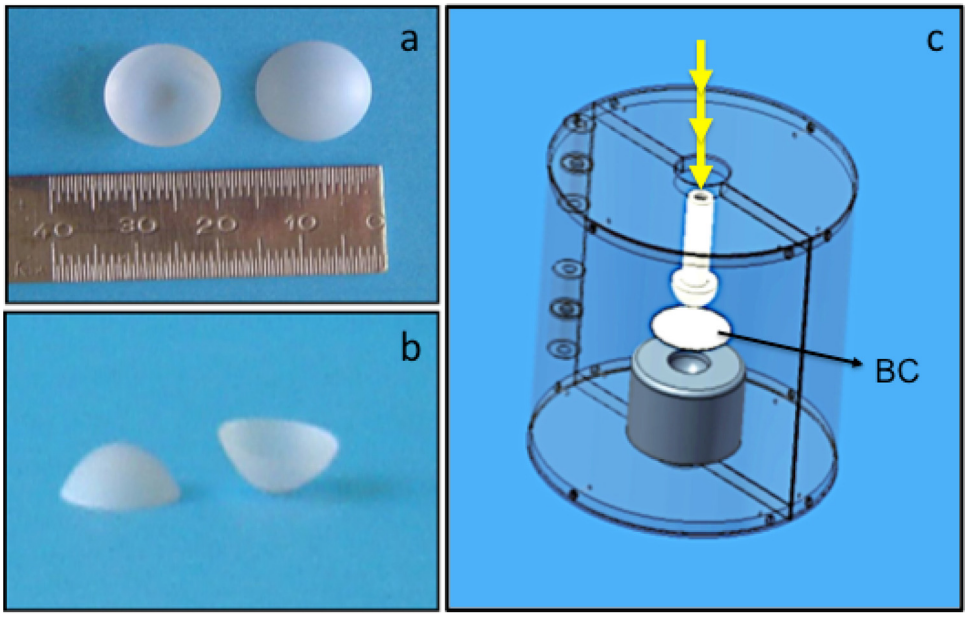

Fig. 5. (a, b) BC contact lens shaped by the designed device and c) device designed to manufacture contact BC lens. The device is constituted by a snip piece which cuts the BC membrane in a round form; a compression piece in the shape of a cylindrical stick made of metal and a base with a semi-spherical undercut at the center made by Teflon. MESSADDEQ, Y., RIBEIRO, S. J. L., THOMAZINI, W. Contact lens for therapy in cases of regeneration of cornea, has bacterial cellulose base. Patent number: PI0603704-6.

was evaluated in a rabbit model. Results demonstrated that 3D porous BC seeded with lingual keratinocytes enhanced urethral tissue regeneration without inducing inflammatory reactions and, at 3 months postoperatively, macroscopic examinations and retrograde urethrograms of urethras revealed that all urethras maintained wide calibers (Huang et al., 2015).

15. Tympanic membrane

Tympanic membrane (TM) perforation is a very common clinical problem resulting into conductive hearing loss and chronic perforations. Acute persistent or chronic TM perforations require surgical interventions such as myringoplasty or tympanoplasty. Current strategies of tissue engineering are focused on treatment of regeneration of TM perforation will probably eliminate the need for conventional surgery. However, it is critical to understand the factors that contribute to the success or failure of TM perforations treatment. As such, several scaffolds and biomolecules have been evaluated for TM tissue engineering. TM regeneration by tissue engineering approach may be considered the greatest advance in otology. BC is presented as an alternative that is safe, biocompatible, and has low toxicity.

Recently, Kim et al. (2013) reported the fabrication of a nanofibrillar patch by using BC as a wound-healing scaffold for TM perforation. BC is endowed with the expected properties of an ideal material for traumatic eardrum perforation repair: a nanostructured surface, biocompatibility, transparency, and appropriate mechanical. *In vitro*, BC nanofibrillar patch promoted the adhesion, proliferation and migration of tympanic membrane cells. Next, *in vivo* assays applying Sprague-Dawley rats demonstrated that the presence of BC patch materials significantly increased the tympanic membrane healing rate as well as recovered the function of tympanic membrane better than spontaneous healing (Kim et al., 2013).

A randomized controlled trial was conducted by Silveira et al. (2016). Forty patients with TM perforation secondary to chronic otitis media were included and randomly assigned in two groups: experimental group (20), treated with BC graft and control group (20) treated with autologous temporal fascia (fascia). The surgical time, hospital stay, time of epithelialization and the rate of TM perforation closure were evaluated and also hospital costs were compared. Despite the closure of perforations were similar in both groups, the average operation time in the fascia group was 76.50 min versus 14.06 min for BC. Finally, a 92% remarkable cost reduction in Brazilian public health system was detected when the hospital costs of fascia group were compared to BC group.

16. Other applications

Regardless many BC applications in the biomedical field, there is still plenty to explore. Noteworthy, Recouvreux et al. (2011) synthesized a large organ-like 3D BC hydrogels with the potential applications in implantable tissue and organ scaffolds such as kidney or liver. Tests in structural characteristics, mechanical properties and biocompatibility are all carried out and a superior performance could be totally expected.

De Souza, Olival-Costa, Da Silva, Pontes, and Lancellotti (2011) conducted an *in vivo* study to evaluate the medialization, inflammatory response, and healing of vocal folds after implantation of a membrane of microbial cellulose in 32 rabbits. The animals received a 0.25 mm^2 membrane of BC in one side of vocal folds and 0.3 cc of distilled water at the other side as represented in the schematic illustration in Fig. 6. The results showed that after 120 days of implantation the material is relatively stable with no major modifications suggesting that BC is a useful material in medial displacement procedures of the vocal folds, as it causes minimal inflammatory reaction and does not extrude.

There is a vast array of literature on the topic of BC nanocomposites matrices and devices for tissue regeneration demonstrating a promising future on new research related to tissue engineering including bioprinting 3D field.

Lately, Nimeskern et al. (2013) designed a BC-based ear-shaped prototype material from the reconstruction of gradient–echo magnetic resonance imaging (MRI). The BC was bioprinted by using a negative silicone mold where the bacteria was guided to reproduce the large-scale features of the outer ear. This study was extremely important to confirm that BC is a promising tissue engineering material with appropriate mechanical properties for ear cartilage

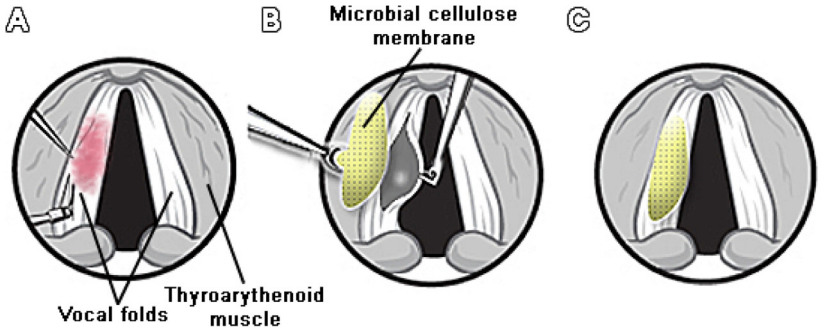

Fig. 6. Schematic drawing of the steps of cellulose implantation showing (A) the scissors opening the thyroid cartilage under direct endoscopic view; (B) the forceps grabbing the cellulose through the thyroid window; and (C) the cellulose positioned lateral to the thyroarytenoid muscle).
Source: Adapted from De Souza et al. (2011).

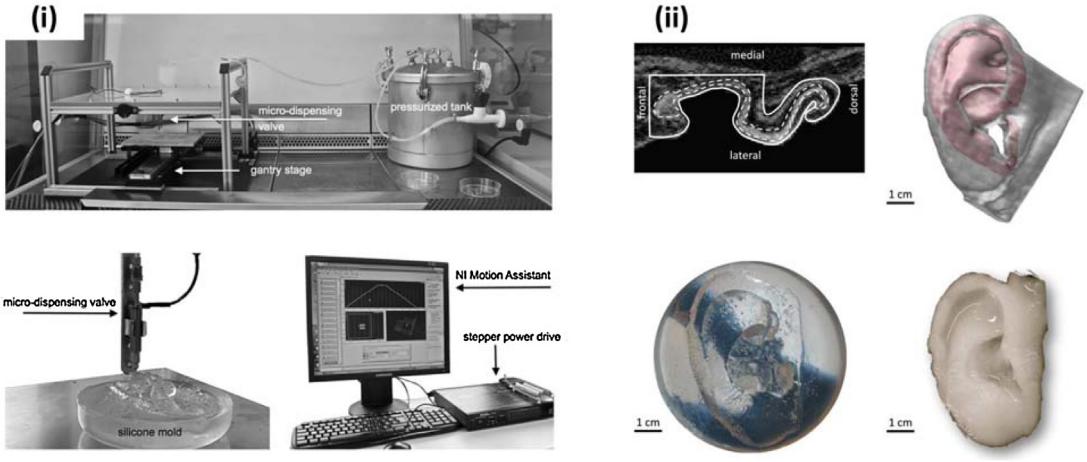

Fig. 7. Biofabrication of a patient-specific ear-shaped BC implant. (i) The bioprinter consists of a high-precision motion system and a microdispensing system. (ii) Transverse slice isolated from a spoiled gradient–echo MRI scan of the volunteer's left ear. The 3D BC implant prototype was fabricated in the shape of the whole outer ear.
Source: Reprinted with permission from Nimeskern et al. (2013).

replacement. Thereby, it may be used to create patient-specific ear shapes as displayed in Fig. 7.

17. Future opportunities and challenges

It is expected that BC and BC based nanocomposites will be more widely applied in biomedical fields due to the unique structure of BC such as high moldability, excellent biocompatibility and exceptional mechanical properties. Herein, we outlined several BC application in different areas of biomedicine. However, there were still some challenges to be overcome with respect to all areas mentioned in this work and many more, such as controlled biodegradation, porosity control, quality consistency, structure difference between surface layer and core area of BC. It is important to point out that more work is expected on BC based nanocomposites due to the high-value-added of functional materials and many novel applications can be expected as current trends. Wherefore, improving properties, reducing production costs and designing a proper industrial fabrication line for BC based nanocomposites are some great examples of main future goals for researchers.

18. Conclusions

Bacterial cellulose is a natural renewable polymer synthesized from the bacterium *Gluconacetobacter xylinus* that is the only known species capable to produce cellulose on an industrial scale. Owning the uniform structure and morphology, BC is free of lignin and hemicellulose and it is endowed with unique characteristics such as high purity, high crystallinity, remarkable mechanical properties, good chemical stability and high water-holding capacity. BC is featured as a completely biocompatible polymer that can be produced in almost any shape due to its high moldability during formation. BC is a really interesting emerging biomaterial being distinctive in several aspects and since its discovery has shown tremendous potential as an effective biopolymer in various fields, because the structural aspect of BC is far superior to those of plant cellulose. Generally, bacterial cellulose has attracted considerable interests in various application fields no matter acting as a matrix or using it in its modified state. Therefore, literature exhibits several biomaterials that were associated with BC, such as collagen, gelatin, fibroin, propolis, chitosan, silver, alginate, hydroxyapatite, BC nanowiskers to reinforce materials, and others, resulting in composites and nanocomposites that were described in detail throughout this article review. Thus, this manuscript revised and presented a great number of different BC-based materials that were designed for biomedical applications (dressings, scaffolds, drug delivery systems), among others. Furthermore, we hope that this review may aggregate high-quality information and may be a benchmark to intensify greater interest of the scientific community in bacterial cellulose and related devices and also inspire the development of new materials in this field.

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