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It is a pleasure for me to introduce an special issue of the *International Journal of Advances in Medical Biotechnology* (IJAMB) covering some of distinctive research papers presented during the *1º Encontro Brasileiro de Biocelulose* (EnBioCel 2018) Araraquara, Brazil. The thematic conference on biocellullose and its applications was organized by the University of Araraquara (UNIARA) on June 4-5, 2018. The conference covered emerging aspects of biocellulose production, properties, modification, main applications and future challenges.

The present special issue comprises seven peer reviewed papers covering the main aspect of bacterial cellulose production and modification with polycaprolactone and phosphates, also the BC most relevant applications in foods, burns, wound healing and drug delivery. In addition, the abstract of 54 selected posters presented during the conference are displayed in this special issue of IJAMB.

Finally, I want to thanks the Editors of IJAMB, Dr. Hernane Barud and Dr. Robson Rosa da Silva, for giving me the opportunity as Guest Editor to publish selected peer reviewed papers presented in the Conference. In addition, I would like to send my appreciation to the reviewers for their invaluable and critical review comments of the manuscripts and extensive to all people that makes this special issue available to the readers.

Welcome to the International Journal of Advances in Medical Biotechnology !

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Bacterial cellulose for food applications

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ABSTRACT

Bacterial cellulose (BC), which is usually produced as pure membranes (sheets) by some bacteria, has been widely studied as a nanomaterial with unique properties for a variety of applications, but it has been actually used mostly for biomedical applications. There are many potential food applications that have not been adequately explored, nata de coco being virtually the only food product from BC on the market. Food applications have usually been considered as less economically feasible, but several studies had demonstrated the suitability of cost-effective fermentation media for producing BC, widening its scope of applications. BC may be used in foods as intact membranes impregnated with other components, or after disintegration or hydrolysis to produce bacterial cellulose

Introduction

Bacterial cellulose production, forms of presentation, and properties

In 1886, Brown[1] reported the production of cellulose pellicles as an extracellular product by *Acetobacter species. Nowadays, b*acterial cellulose (BC) is well known as a nanostructured exopolysaccharide produced by some bacteria, especially from the genus *Komagataeibacter* (former *Gluconacetobacter*), in carbon- and nitrogen-containing media.[2] When compared to plant cellullose, BC has the advantage of not requiring harsh chemical treatments for its isolation,[3] since it is free from lignin and hemicelluloses. Moreover, it exhibits higher crystallinity, lower density, higher water holding capacity, and higher tensile strength due to its web-like network structure.[4,5,6] BC is usually produced under static conditions as a membrane (pellicle), but it may be also produced under stirred conditions as fibrous BC [2,7] or as small pellets.[7,8]

Most BC applications have been focused on biomedical applications, including artificial skin and blood vessels, wound dressings, and drug carriers.[2,6] Food applications have traditionally been considered as less interesting, probably because of a perceived incompatibility between the usually low price of food products and the high cost of producing BC, including the costly traditional Hestrin and Schramm (HS) medium. However, such perception has changed since a number of studies had demonstrated the feasibility of producing BC by using cost-effective fermentation media such as fruit byproducts,[9,10,11,12] distillery wastewater,[13] and glycerol from biodiesel production.[12] Moreover, BC is categorized as "generally recognized as safe" (GRAS) by the Food and Drug Administration.[3] A recent review on toxicological data on BC[14] have corroborated its safety for food applications.

BC membranes may be used for food applications in different ways (Figure 1). Intact (or cut) BC membranes may be soaked in solutions or dispersions in order to be impregnated with sugars (as for nata), pigments or pigment-producing molds,[15] and antimicrobials.[16,17] For applications which require BC as a powder or suspension prior to formulation, the membranes are disintegrated by chemical and/or physical methods to produce bacterial cellulose nanofibrils (BCNF), or acid hydrolyzed to obtain bacterial cellulose nanocrystals (BCNCs).[18]

This article presents a brief overview of actual and potential applications of bacterial cellulose in food industry, based on the remarkable properties of such a unique natural nanomaterial.

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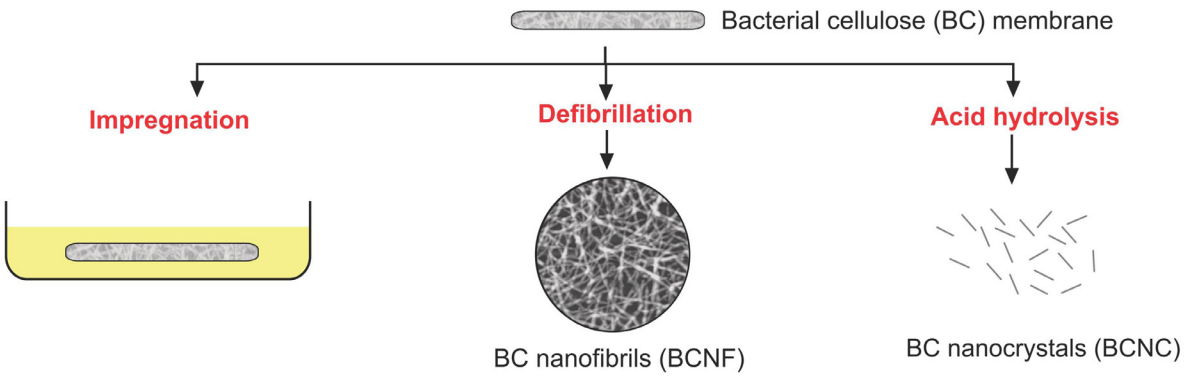

Figure 1 - Forms of applications of bacterial cellulose.

Nata

The most traditional food use of BC is as the raw material for *nata* (or *nata de coco*), a traditional dessert from the Philippines that consists of cubes cut from BC sheets produced with coconut water[4] or coconut milk,[19] and immersed in a sugar syrup; similar products such as *nata de piña* (produced from pineapple juice) or nata de soya (from tofu whey) may also be prepared.[4,20] Actually, any widely appreciated fruit juice could be tested for the development of novel nata products, adding regional appeal to the traditional Asian product.

The Philippinean production and exportation of *nata* have declined in the last few years. The highest volume (about 6300 ton) and value (about US$ 6.6 billion) of *nata* exportation by the country was recorded in 2011.[19] The Philippines are still the main nata-de-coco producer, mainly by small scale producers; however, the country has faced problems, as described by Piadozo[19], including: (a) the high cost of the raw materials (especially coconut milk), which has been aggravated by a massive infestation of coconut trees by the coconut scale insect in some producing regions, (b) the adverse effects of extreme temperatures on the bacteria activity and nata quality, and (c) competition from other coconut-producing neighbor countries, which have improved the process and the product quality.

The effects of pH changes, added salt, heating and freezing on the physical properties of *nata* were evaluated.[21] Freezing (-20°C, 24 h) has been reported to increase the elasticity of the material, while heating (100°C, 3 h) did not significantly affect nata texture. NaCl addition increased the material hardness, unless when nata was previously acidified (or alkalinized), indicating that a pH change is required to prepare salted nata products with a suitable texture.

Colored nata was obtained by Sheu et al,[15] who soaked BC in a solution inoculated with *Monascus purpureus,* a mold that produces yellow, orange and/or red pigments. Since the mold also produces a cholesterol-lowering agent (monacolin K), *Monascus-nata* products have been presented as novel functional foods,[22] and could be the base for an artificial-vegetarian meat substitute.[23]

Edible sheets or films

Our group has recently produced edible films from BCNF and/or pectin (in different proportions) added with mango or guava purees.[24] Films with more BCNF than pectin tended to be stronger and with improved water resistance and water vapor barrier. The films could be applied as edible primary packaging, food wrappings, or even consumed by themselves as fruit sheets or ribbons, having a "fiber-rich" appeal. Alternatively, the sheets could have been prepared by impregnation of BC membranes into fruit juices or nectars.

When applied as edible packaging films, the materials could also include active compounds (such as antimicrobial or antioxidant agents) to help extend the shelf stability of the food to be packed. Some studies have reported effective antimicrobial actions of BC films containing food-grade antimicrobials such as nisin[16] and lactoferrin.[25] Antibacterial sausage casings have also been prepared from BC tubes impregnated with ε-polylysine, extending the stability of the sausages when compared to the control without ε-polylysin.[17]

Fat replacer

BC has been used as a fat replacer in Chinese-style meatballs.[26] When BC was added at 20% (completely replacing the added fat of the product), the cooking losses were higher than those of the control, and the texture was softer, which resulted in decreased acceptance. On the other hand, meatballs with 10% added BC presented similar sensory acceptance and stability when compared to the control.

A Surimi product prepared by using BC as a fat replacer presented increased gel strength and water-holding capacity due to its enhanced network structure.[27]

Moreover, being a dietary fiber, BC offers a number of health benefits. Chau et al.[28] not only have demonstrated the hypolipidemic and hypocholesterolemic effects of BC in hamsters, but reported that its lipid- and cholesterol-lowering efficacy was significantly higher when compared to those of plant cellulose. Moreover, rats fed with meals

containing BC presented increased fecal weight and decreased transit time than rats from the control group.[29]

Texture modifier

Because of its structural properties, BC may be used for several applications related to texture modification, including thickening, gelling, and water binding.[20] An ice cream containing BC was reported to retain its shape for 60 min at room temperature, because of the water binding properties of BC, while control ice cream (without BC) was completely melted after the same 60 min.[30] Other texture-modification applications have been reported, such as gel strengthener for Tofu, preventer of cocoa precipitation in chocolate beverages, and stabilizer of beverage viscosity upon a heat treatment.[30]

Pickering emulsion stabilizer

Pickering emulsions are those stabilized by solid colloidal particles that adsorb onto the oil-water interfaces, forming a strong monolayer that avoids coalescence, even without the presence of surfactants.[5] An amphiphilic character is typically required of the solid particles, although it is not mandatory.[31] Advantages of Pickering emulsions (when compared to conventional emulsions stabilized by surfactants) include reduced foaming problems, lower toxicity, and reduced environmental impacts.[32]

Cellulose is considered as amphiphilic, since it combines the hydrophilicity derived from the high surface density of hydroxyl groups and the hydrophobic interactions resulting from the crystalline organization and extensive hydrogen bonding of chains.[31,32]

Both BCNF and BCNC have been applied to stabilize surfactant-free Pickering emulsions.[31,32] Actually, BC was more effective to stabilize oil-in-water (o/w) emulsions than commercial cellulose derivatives (HPMC and CMC), which was attributed to its strong fibril network adsorbed to the oil droplets.[5] BCNCs (produced by sulfuric acid hydrolysis) were reported to perform better than BCNFs to stabilize o/w emulsions, which was ascribed both to their smaller size and to their higher (in absolute values) zeta potential derived from the sulfate groups.[32]

Immobilizer for probiotics and enzymes

Probiotic bacteria have been demonstrated to balance gut microflora and to provide consumers with health benefits. In the last decades, they have been used in several food applications. However, the survival of probiotics is usually impaired by processing and storage conditions, as well as by transit through the gastrointestinal tract.[33] BC has been demonstrated to be a suitable cryoprotectant and immobilization support for probiotic bacteria,[34] protecting the bacterial cells against gastric juices and thus favoring probiotic viability.[35]

BC has been also suggested to immobilize enzymes such as laccase,[36] which may be a useful approach to improve yield, quality and/or stability of foods.[23]

Final considerations

Although bacterial cellulose is a GRAS polysaccharide material with unique properties such as water resistance and high water retention capacity, it has not yet been suitably explored for its potential food applications. Nata is basically the only bacterial cellulose product at the food market, whereas a variety of other promising applications deserve to receive serious consideration.

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Mini Review

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Bacterial cellulose: Application as drug delivery system

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ABSTRACT

Bacterial cellulose (BC) is a very interesting biopolymer to the biomedical application, including drug delivery system, due to unique characteristics as such as high degree purity and of porosity, relatively high permeability to liquid and gases, high holding water capacity, tensile strength, and randomly oriented three-dimensional fiber network. Several authors described the use of BC membranes or copolymers to use as drug delivery system. The aim of the present mini-review was to show the wide and vantages application of the BC and its copolymers for use as controlled drug delivery system.

Introduction

Polymeric drug delivery systems may be designed in many forms, including matrices, composites, pure membranes and copolymers in which the bioactive compound must be dispersed or dissolved (1,2). The route of administration, carrier formulation, release mechanism and physicochemical properties of the drug molecule may influence the rate of release and, therefore, should be considered when selecting a suitable polymer for this purpose (2,3). In addition, the polymers used for the development of drug delivery systems must be chemically inert and present appropriate physical and chemical characteristics (2). In the last years, BC (Figure 1), a very interesting biopolymer, has been widely applied in transdermal drug delivery as membranes, being commonly used in the fabrication of matrix-type patches due singular properties (4), such as high degree purity and of porosity, relative high permeability to liquid and gases, high holding water capacity, tensile strength and randomly oriented three-dimension fibers network, viscoelasticity and poroelasticity (5,6).

BC can be produced by several organisms species, (7,8) with special emphasis to cellulose produced by bacteria of the genus *Gluconacetobacter*, recently named *Komagataeibacter* (Figure 2), especially the species *K. xylinus* and *K. hansenii* (6,9,10), using a variety of natural and synthetic culture media with several carbon sources of different origins (11,12). Nevertheless, BC membranes maintain a physical barrier that reduces pain, bacterial infection and allows drug transfer into the wounded region (13–15). These BC membranes characteristics constitute an important aim to development of studies for application this biopolymer as drug delivery system (16–18).

The aim of the present mini-review was to show the wide and vantages application of the BC and its copolymers for use as controlled drug delivery system.

Application of bacterial cellulose as a drug delivery system

In recent years, several drug delivery systems based in BC membranes for various pharmaceutical applications have been proposed including antimicrobial, and anticancer agents, small molecules, inorganic nanoparticles and a metal complex (19).

Studies developed by Stoica-Guzun et al. (20) demonstrated the delivery of the antibiotic tetracycline encapsulated on BC matrix comparing irradiated (doses of 5 or 15 kGy) to non-irradiated BC membranes an *in vitro* study demonstrating that electron beam radiated over BC-tetracycline system promoted faster drug release rate.

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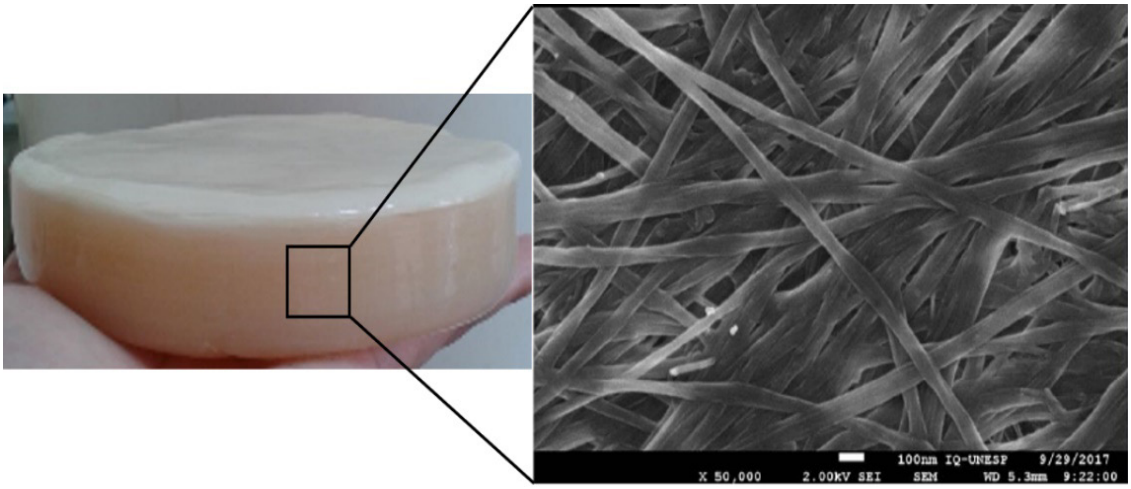

Figure 1 – Swollen BC membrane and Scanning Electron Microscopy (SEM) BC membranes randomly oriented three-dimension fibers network (50.000x)..

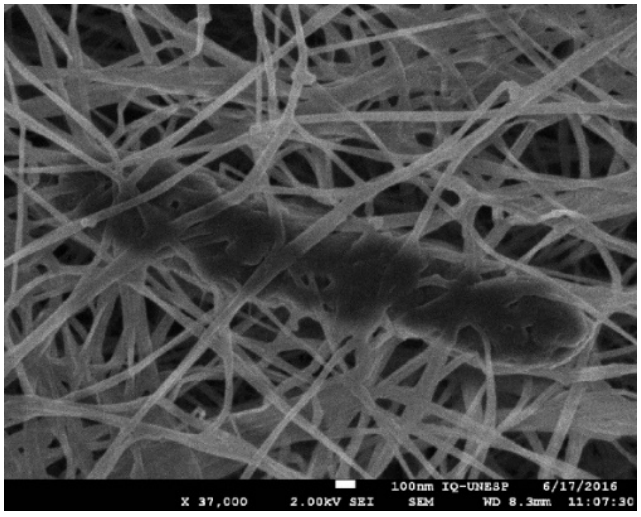

Figure 2 - Scanning Electron Microscopy (SEM) *G. hansenii* ATCC 23769 (37.000x)

Antimicrobial bacterial cellulose-silver nanoparticles composite membranes obtained by *in situ* preparation of silver nanoparticles from hydrolytic decomposition of silver nitrate solution using triethanolamine as reducing and complexing agent exhibited strong antimicrobial activity against Gram-positive *S. aureus* and Gram-negative *E. coli* and *P. aeruginosa* bacteria Gram-positive *S. aureus* (21).

In another work, Kaplan et al. (22) performed a comparative study to evaluate the in vitro release behavior of gentamicin (GM) and ampicillin (AMP) by BC membranes. These authors demonstrated that membranes exhibited sustained release capacity of AMP and GM in 7 days and the amounts of antibiotic released by BC reached the proportion of dose required to inhibit the growth of *E. coli*, *E. feacalis*, *S. aureus* and *P. aeruginosa.*

In study using the Box-Behnken statistical design to study the release of amoxicillin (AMX) from the BC, BC / glycerol and BC / hexadecyltrimethylammonium bromide enhancer showed that amoxicillin concentration had a greater influence on drug release and a significant contribution was also observed for the linear and quadratic terms of the glycerol concentration, the linear concentration of potentiator, and the interaction between the concentration of glycerol and concentration of the enhancer. These results show that independent variables affevct the release of AMX from BC membranes (23).

In vitro antibacterial assay using BC composite membranes prepared with tetracycline hydrochloride (BC-TCH) demonstrates that this composites displayed excellent antibacterial activity solely associated with the loaded TCH drug (24).

A study using immobilized lysozyme onto BC nanofibers (BCNF) produced by physical absorption method was performed to evaluate the antimicrobial activity and other properties of immobilized lysozyme and also morphological characteristics of BCNF. This result demonstrates that the antimicrobial activity of lysozyme against *S. aureus*, *E. coli*, *L. monocytogenes*, *Y. entrocolitica*, *Aspergillus niger,* and *Saccharomyces cerevisiae* were increased after immobilization evidencing the potential for the use of BCNF as lysozyme delivery system (25).

Transparent antimicrobial silver nanoparticles/bacterial cellulose (AgNPs/BC) membranes produced by reducing silver nitrate as a precursor in the presence of sodium tripolyphosphate and *in situ* impregnation into the BC membranes. The AgNPs/BC membranes were nontoxic and showed good biocompatibility on peripheral blood mononuclear cells due to the controlled silver ion release. According to the results, it is suggested that the AgNPs/BC membranes can be applied for many antimicrobial purposes such as antibacterial wound dressing (26).

In a study, using bilayer BC membrane produced by *G. hansenii* ATCC 23769, from sugar-cane molasses carbon sources, impregnated with ceftriaxone (CRO), was demonstrated a higher capacity for retention and release of CRO when compared to the commercial BC membranes (18).

In a recent study, Volova et al. (27) demonstrated

pronounced antibacterial activity against *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *S. aureus*, and the BC/ antibiotics amikacin and ceftriaxone composites were more active than BC/AgNp. *S. aureus* was the most susceptible to the effect of BC composites.

Lazarini et al. (18), obtained a dissimilar BC membrane with high drug delivery capacity by *G. hansenii* variety achieve after application of different culture temperatures. The BC membrane produced by variety acquired from the culture at 35º C produced membranes with dissimilar degrees of interweaving and fibers thickness and high dry mass yield. This BC obtained was impregnated with CRO and maintained release capacity for 72 hours.

A new hybrid material based on bacterial cellulose containing silver phosphate microparticles on one side of the matrix and high ciprofloxacin loading has been developed by Bayón et al. (28). The AgP-MPs developed by the self-assembly technique on only one side of the bacterial cellulose surface provide a novel and promising composite material with excellent antimicrobial activity against both Gram-positive *S. aureus* and Gram-negative *E. coli* bacteria. The BC membranes obtained showed relevant properties such as non-adhesive hydrogel dressing capability, high skin tissue compatibility, excellent water uptake ability, high mechanical strength, and air permeability.

In a study designed to prepare surface modified BC matrices by treatment with acetic anhydride, freeze drying, and oven drying, the BC was loaded with model drugs selected based on their aqueous solubility, faintly water-soluble famotidine and highly water-soluble tizanidine. The chemical structure, the concentration of the drug loaded, the concentration of the surface modifier and the modifications pre and post-loading of the drug altered the physicochemical properties of the BC matrices, which in turn affected the drug release behavior. The obtained results demonstrated that the surface modifications were found to be effective for controlling the drug release properties demonstrating the potential these BC matrices for applications as modified drug delivery system (29).

Results obtained in recent study, development by Lima Fontes (30), BC/ carboxymethylcelullose (BC/CMC) biocomposites with different DS-CMC (DS from 0.7 to 1.2) were loaded with methotrexate (MTX), drug used treatment of psoriasis, an autoimmune disorder of skin, in order to evaluate their impact as a drug delivery system. All samples showed a typical burst release effect in the first 15 min of a test, however, the BC/CMC (DS0.9) biocomposite promoted a slight lowering of MTX release rates, suggesting that the DS of CMC can be considered the key factor to modulate the BC properties.

A novel hybrid biomaterial composed bacterial cellulose hydrogel and nanostructured lipid carriers (NLCs) for application as local drug delivery implant for cancer therapy using doxorubicin (Dox) as drug model were described by Cacicedo et al. (31). NLCs loaded with cationic Dox (NLCs-H) or neutral Dox (NLCs-N) were fully characterized and their cell internalization and cytotoxic efficacy were evaluated *in vitro* against MDA-MB-231 cells. Both NLCs internalized via the endocytic pathway while allowing a sustained release of the Dox, which in turn rendered IC50 values below of those of free Dox. Thereafter, a combination of NLCs-H and NLCs-N loaded into BC (BC-NLCs-NH) was analyzed *in vivo* into an orthotopic mouse model. BC-NLCs-NH showing a significant reduction in tumor growth, metastasis incidence and local drug toxicities. These results demonstrate the potential use of BC-NLCs-NH as local drug delivery system.

Conclusion

After reviewing published data, it was evident that BC membrane and copolymers showed high potential pharmaceutical use with advantage application for the controlled drug delivery system justifying the increase of the interest of the researchers in the development of products based in BC to this application.

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Recent advances in methods of synthesis and applications of bacterial cellulose/calcium phosphates composites in bone tissue engineering

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ABSTRACT

Bacterial cellulose (BC) is a nanofibrous biomaterial biosynthetized by a series of acetic bacteria with unique properties with application in many tissue engineering purposes. Calcium phosphates (CPs), mainly hydroxyapatite, are bioceramics that possess similar composition of host bones and are able to stimulate osteoconduction and osteointegration to living tissues.

Bacterial cellulose-calcium phosphates composites have caught the attention of researchers by their excellent mechanical properties and biocompatibility, being considered an excellent proposal to development of new synthetic grafts to bone tissue engineering. The minireview presented here focuses on various fabrication methods used to prepare and novel applications of BC-CPs composites and their applications in BTE.

Introduction

In the last decades, researchers of different fields, such as materials science and engineering, orthopedics and dentistry, have made efforts to develop new strategies in order to replace autologous, allografts and xenografts-based therapies for new viable alternatives to solve the problem of millions of people that suffer with trauma, tumors and bone related diseases. Despite having many advantages, as stimulate osteoconductive, osteoinductive and low immune responses, autologous bone grafts, considered gold standards in orthopedic and dentistry fields, provide a limited supply of implant required for surgery. Furthermore, a patient that requires this procedure is submitted to subsequent harvesting and insertion of material in the fractured area, that leads to considerable incidence of site morbidity associated with the harvested graft.[1-4]

Allografts and xenografts, although are commonly used as alternative techniques to overcome some disadvantages of autograft therapy, due to unlimited availability of material and no occurrence of donor sites morbidity (since the grafts are harvested from bone banks), are limited because of the possibility of infection and immunological rejection. Alloplastic grafts obtained from synthetic or natural biomaterials has been considered a promisor solution to obtain biocompatible and well-integrated materials with host bone, stimulating bone regeneration, that is the main goal of bone tissue engineering (BTE)[3,5-7].

Tissue engineering (TE) is a crucial subfield of regenerative medicine, whose major concern is manufacturing parts of body, such as tissues and organs *ex vivo.* Cells culture in scaffolds and the monitoring of their proliferation, differentiation and activity is an essential concept of TE. The main goal is to develop living constructs using biomaterials, cells and growth factors to restore, regenerate, preserve or enhance functions of damaged or lost tissues [8-10].

From cell cultures in scaffolds, the next step is to implant the bioengineered organs or tissues in humans to provide their integration and the biological environment to synthetize extracellular matrix (ECM). The main requirement of a TE scaffold is that degrades over time, while allows tissue regeneration. Several materials have been successfully used for TE and specifically in BTE applications.[11-12]

Bone is a hard tissue that support the structure of body. It presents a three-dimensional (3D) hierarchical structure compound by cells, non-collagenous proteins, hyaluronan, peptoglycans and nanocollagen fibers mineralized with hydroxyapatite (HA). HA, in particular, is the major bio-

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active inorganic material of bone and is able to support bone growth and osseointegration, being used in orthopedic, dental implants, spinal fusion and treatment of bone defects. HA/collagen composites have been traditionally employed to produce artificial bones.

Nevertheless, a more recent trend is to use other biocompatible materials to replace collagen, due to the possibility of cross-infection and poor definition of commercial sources[13-14].

Bacterial cellulose (BC), a renewable nanobiomaterial with a three-dimensional structure of cellulose fibers, may be considered a good candidate for this purpose, once it has been proved that BC scaffolds pores are able to support the ingrowth of human chondrocytes and human smooth muscle cells *in vivo* [13,15].

Some studies have described the manufacturing and applications of BC-CPs composites for bone tissue engineering purposes, especially by enhancing osteoblastic cell proliferation and differentiation. In this context, the present review focuses on studies related to different methods of synthesis and applications of BC/CPs composites in bone tissue engineering and hence in regenerative medicine in the last ten years[16].

Bacterial cellulose: a promisor material for TE

Bacterial cellulose (BC), also known as biocellulose or microbial cellulose is a biocompatible polysaccharide biosynthesized by several species of bacteria including those belonging to the genera *Komagataeibacter* (formerly *Gluconacetobacter*), *Aerobacter, Agrobacterium, Zooglea, Azotobacter, Achromobacter, Alcaligenes, Acantamoeba, Rhizobium, Pseudomonas, Salmonella* and *Sarcina*[17-18].

This biomaterial presents the crystalline form cellulose I and chemical structured formed by linked linear chains of β-1,4-glucopyranose residues, that are the same of plant -based cellulose[19-20]. However, bacterial cellulose is free of lignin and hemicelluloses and presents high purity and remarkable mechanical and physical properties such as high elasticity, durability, resistance to traction and high ability to retain and absorb water. BC is also biodegradable and easily purified using NaOH solution[21-22]. All those unique and advantageous properties are mainly derived from its ultrafine network structure (with approximately 1.5 nm in width) and enable BC to be applied in various fields such as medicinal (TE), environmental, food and cosmetics purposes[23].

Hydroxyapatite and other calcium phosphates for biomedical purposes

Calcium phosphates (CPs) are the main constituents of mineral phase of bones and teeth in vertebrates. For this reason, synthetic CPs have osteoconductive and osteoinductive properties and have been widely used for bone tissue regeneration and augmentation. CPs as biomaterials are generally classified according to composition as calcium hydroxyapatite (HA), Ca10(PO4)6(OH)2; alpha- or beta-tricalcium phosphate (α- or β-TCP), Ca3(PO4)2; biphasic calcium phosphates (BCPs) for mixtures of HA and β-TCP; and unsintered apatites or calcium-deficient apatites (CDAs)[24-28]. Solubility and biological properties of those materials are extremely dependent on crystal size, ionic impurities, specific surface area, and both macroporosity and microporosity. Cell colonization, for example, is possible in those CPs materials since it is induced by associating CPs with organic substances which are calcined before sintering to achieve convenient porosity[29-31].

Despite having exceptional characteristics, such as a good integration with the host bone tissue and make the environment more favorable for bone regeneration, due to their cytocompatibility, synthetic CPs commonly present poor biomechanical properties arising from their weak tensile strength and inherent brittleness. To overcome this limitation and enable clinical applications, an alternative solution has been to add internal or external factors or even to associate synthetic or natural polymers with CPs forming composites[16].

BC-CPs composites for BTE applications: methods of synthesis and applications

BC fabrication, as noted above, is resulting of the activity some kind of Gram-negative acetic bacteria. There are two methods to fabricating BC depending on the purposes, namely as stationary and agitated culture. Pellicles are formed under static culture, by the accumulation of a gelatinous membrane at the air/liquid interface and fibrils or spherical-like particles are obtained under agitated culture conditions[19,32].

Considering the macroscopic morphology of the resulting composite, there are three pathways to prepare BC/HA composites, which can be extended to BC/CPs composites: (i) *in situ* growing of CPs into culture media; (ii) *ex situ* synthesis of CPs in BC fibers or for physical mixing of those two materials; (iii) synthesis of BC composite from BC solution. Fig. 1 presents a schematic representation of each approach for prepare those composites[16].

The *in situ* approach consists of adding the reinforcement CPs material into BC culture media at the beginning of BC cultivation in agitated or static conditions (Fig. 1a). This process has a great advantage of encaging materials that become part of the fibrils, modifying and enhancing substantially the physico-mechanical properties of BC fibrils, however presents a critical limitation when involves the incorporation of reinforcement materials that also have antibacterial activity against BC strains. From this point of view, CPs are suitable to be incorporated into culture media, since some studies have revealed that HA, for example, has been successfully suspended before BC cultivation without toxic effects against BC producer strains. Another disadvantage is related with the short time in which those particles remain suspended in BC synthesis media. To overcome this problem, the researchers have

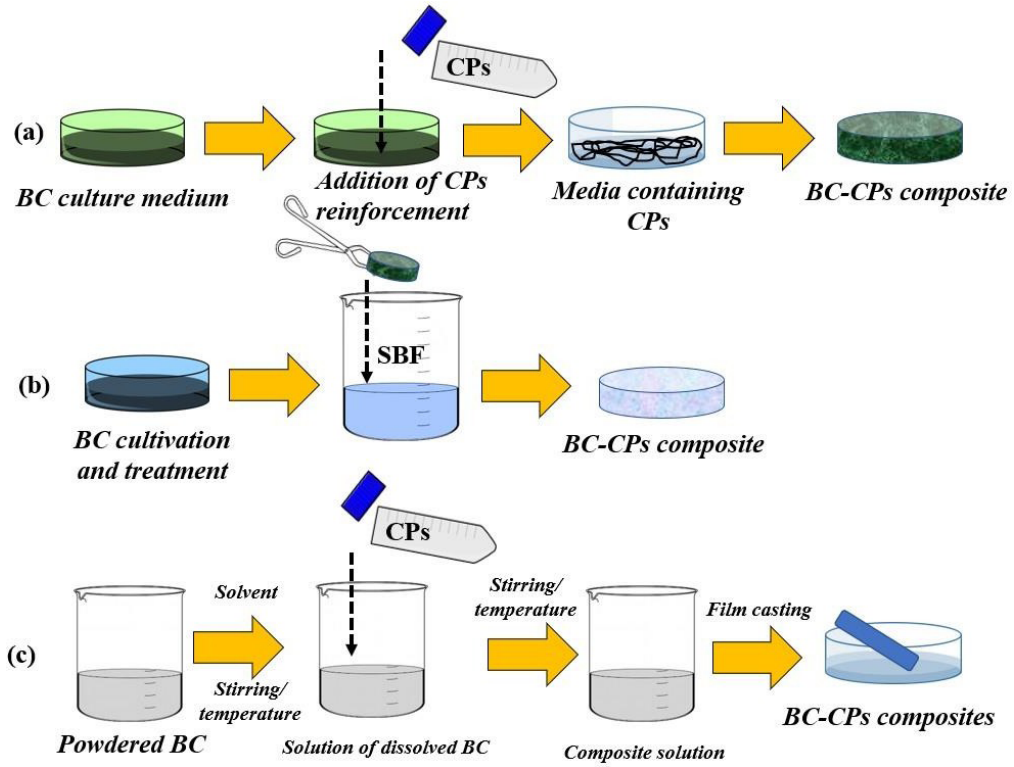

Fig. 1. (a) Schematic representation of BC composites produced through in situ synthetic strategy. Particles entrapped between growing BC fibrils. (b) Schematic representation of BC composites synthesized through an ex situ synthetic strategy. (c) Schematic representation of BC composites synthesized from dissolved BC solutions. The composite solution is casted to prepare BC films.

proposed strategies such as BC synthesis in agitated conditions or developing vessels equipped with spinning discs. Agitation culture, as discussed before, produces fibrils and cannot be applied to produce gels or sheets used in biomedical applications[16,33].

The *ex situ method*, as noted in Fig 1b, involves the incorporation of liquids or nanoparticles into BC structural matrix. The application of this method is successful when the reinforcement materials incorporated have suitable size and reactivity to the group OH of BC.

For this reason, only submicron and nanoparticles can be entrapped through hydrogen bonds with OH group in the BC matrix for this strategy, being an efficient and simpler method to obtain sheets and gels from static cultures.

The synthesis of BC composites from BC solutions is the best method to fabricate a large variety of different composites with more control of BC matrix composites and reinforcement materials. One of the most critical challenges is to find a suitable solvent to solve BC membranes and generally those solvents cause damages to BC structure. We summarized in Table 1 the most recent studies in which those strategies are employed to produce BC-CPs composites and the main findings of each one. These studies were found by combining the keywords "bacterial cellulose" and ("calcium phosphate" or hydroxyapatite) in *Web of Science* database in June 2018.

According to those reports, it is noted that in most studies, *ex situ* strategy has been chosen to prepare BC-CPs composites. In this approach, CPs are incorporated after BC cultivation process by soaking BC membranes in simulated body fluid (SBF) with an ion concentration equivalent to human blood plasma at physiological conditions of pH and temperature to stimulate biomineralization or in alternate solutions of Ca^{2+} and $PO4^{3-}$ ions[16,48].

Among the surveyed studies, Grande et al.[47] and Romanov et al.[44] were the only ones to relate the *in situ* addition of HA to BC culture medium in order to produce BC-HA composites. Both of them previously synthetized HA via a wet chemical precipitation and then the apatite was incorporated to the culture medium. However, considering that the viscosity of the culture medium was not ideal to suspend homogeneously HA particles, Grande et al.[47] modified it, by adding carboxymethylcellulose (CMC) at 1 or 2 % (w/v). The incorporation of CMC in the culture medium, during the formation of cellulose fibrils caused a decrease of almost 50% in average diameter of cellulose fibrils and an increase of 47.8% in pore size. Also, in accordance to the authors, an amount of 22% of HA was not entrapped in BC nanocomposite.

Romanov et al.[44], in turn, have suspended HA in BC culture medium without adjusting of viscosity and according to their results, have had the formation of partly textured HA on the surface of the cellulose fibrils. Actually, in this study, other two methods have been

used to prepare BC-HA composites. In order to implement them, HA was suspended in the process of dispersion of BC nanogel films or synthetized in the suspended BC medium. All those methods of BC-HA have produced materials with potential biomedical applications, although their biological properties were not assessed.

Several studies have been carried out with BC membranes *in vitro* in preclinical assays for biomedical purposes. These studies have investigated the application of BC in drug, hormone and protein release systems, artificial skin, wound dressing, artificial cartilage, menisci, invertebral disks, dental implants valvular prostheses, artificial cornea and the urethrata[49-54]. Therefore, the researches involving the use of BC membranes for tissue engineering applications have increased considerably in number, over the last few years, according to the Fig. 2. These research works are based on bacterial cellulose in tissue engineering and bacterial cellulose/calcium phosphates composites.

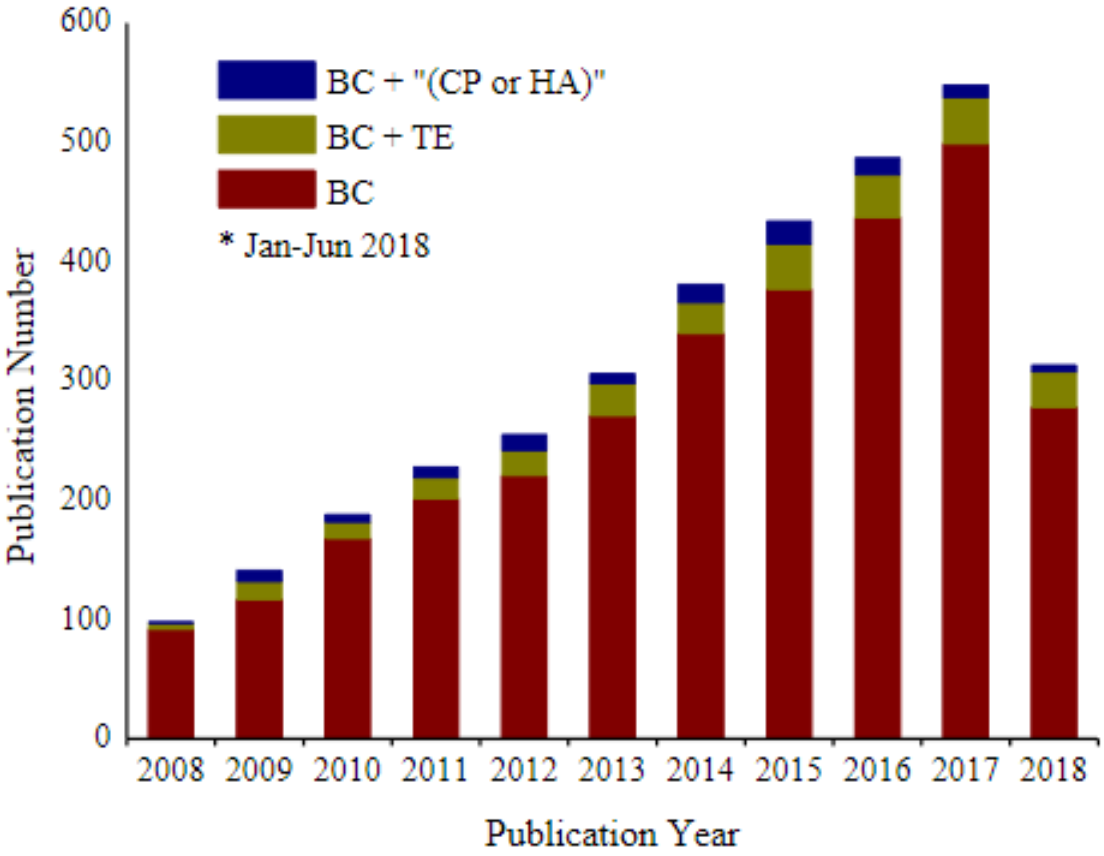

Fig. 2 - Annual publications of BC, BC with TE applications and BC-CPs composites since 2008 to June 2018. The search was carried out in *Web of Science*^{TM}.

BC-CPs composites	Findings
BC-HA composites using silk fibroin as reinforcing phase	Silk fibroin from two different species was inco composites. Bacterial cellulose-*Antheraea yam* *Bombyx mori* silk fibroin/hydroxyapatite and cytocompatibility, being the most indicated for
BC-HA composites produced *ex situ*	BC was firstly modified with PVP and after activ produce BC-HA. In order to mimic cartilage tissue and *in vitro* proliferation of osteoblasts and hA bilayer scaffolds accelerated the regeneration o
BC-gelatin/HA composites	BC-gelatin/HA presented rougher surface topog with BC/HA composites and *in vitro* cell cultu gelatin/HA revealed better adhesion, proliferati
BC modified with chondroitin sulfate (added in culture medium).	BC was produced with addition of chondroitin su be applied to guided bone regeneration.
BC-β-TCP-HA based hydrogel scaffolds with addition of CMC or PVP to culture media.	BC based hydrogels were produced by addin suspension and after that suspending β-TCP an and an efficient interaction between the implan technique.
BC-HA composites with adjust of mechanical properties	BC gel-film was firstly produced. The BC-HA com properties of the resulting composites. The auth
Calcium phosphates grown on BC as template	In this report, the authors proposed the use o deposited by soaking/ultrasound technique. CP
Modification of BC culture media with hyaluronic acid 1% (w/w) and *ex situ* biomimetic precipitation of CPs	BC fermentation medium was modified with hy favor biomedical applications. After that, biomi for perspectives in dental materials scaffolds ap
3D nanofibrous BC-based templates with varying surface chemistry	BC pellicles were produced and its surface was m each modified surface was investigated by X-ray
BC-HA associated with bone growth peptides (OGP) or pentapeptide OGP (10-14)	BC-HA, BC-HA-OGP and BC-HA-OGP(10-14) wer calvarial defects. It was verified that BC-HA was
Modification of BC culture medium by adding chondroitin and hyaluronic acid (1% w/w) and *ex situ* biomimetic precipitation of CPs	BC fermentation medium was modified with cho of CP was performed from simulated body fluid
BC-HA composites synthetized by different techniques	BC-HA composites were produced by combined in the culture medium (*in situ*) and synthesis of

Fig. 2 - Annual publications of BC, BC with TE applications and BC-CPs compo

	Reference
orporated to BC membranes to improve mechanical properties of BC-HA *amai* silk fibroin/hydroxyapatite had advantages over Bacterial cellulose-Bacterial cellulose/Hydroxyapatite on mechanical strength and *in vitro* applications in BTE.	[34]
vated with $CaCl_2$ 0.1 mol.L^{-1} for three days to be soaked with 1.5 SBF and e, BC was treated with chondroitin sulfate salt sodium (BC-GAG). Attachment ACs were supported, respectively. *In vitro* studies revealed that those BC f articular cartilage and subchondral bone in a rat model.	[35]
graphy, higher thermal stability and mechanical strength when compared re of rat bone marrow-derived mesenchymal stem cells cultured in BC-on and differentiation than the cells cultured in BC-gelatin.	[36]
ulfate with good *ex situ* calcium phosphate deposition. Those materialscan	[37]
ng polyvinylpyrolidone (PVP) and carboxymethylcellulose (CMC) to BC d HA. $CaCO_3$ was then incorporated in order to achieve biomineralization nt and the host bone. Hydrogels scaffolds were produced by solvent cast	[38]
posites were prepared *ex situ*, increasing BC fraction to modify mechanical nors proposed the use of those materials for bonereplacement/fillers.	[39]
f BC previously cultivated in static conditions as a template to grow CPs s were non-toxic and suggested to be applied as cements/fillers.	[18]
yaluronic acid 1% (w/w) and irradiated with gamma radiation in order to metic precipitation of CP was performed from simulated body fluid (SBF), plications.	[40]
odified by coating on it various materials. Calcium phosphate formation for absorption near-edge structure (XANES) spectroscopy.	[41]
e assayed to evaluate their potential in bone regeneration in critical- size efficient for bone regeneration in critical-size conditions.	[42]
ndroitin and hyaluronic acid 1% (w/w). After that, biomimetic precipitation (SBF), for perspectives in dental materials scaffolds applications.	[43]
aggregation of HA and BC suspensions, introduction of the HA suspension HA in the medium of dispersed cellulose.	[44]

►►

osites since 2008 to June 2018. The search was carried out in *Web of Science*^{TM}.

BC-HA composites with adjust of mechanical properties	BC gel-film was firstly produced. The BC-HA com properties of the resulting composites. The autl
Calcium phosphates grown on BC as template	In this report, the authors proposed the use o deposited by soaking/ultrasound technique. CP
Modification of BC culture media with hyaluronic acid 1% (w/w) and *ex situ* biomimetic precipitation of CPs	BC fermentation medium was modified with hy favor biomedical applications. After that, biomi for perspectives in dental materials scaffolds ap
3D nanofibrous BC-based templates with varying surface chemistry	BC pellicles were produced and its surface was m each modified surface was investigated by X-ray
BC-HA associated with bone growth peptides (OGP) or pentapeptide OGP (10-14)	BC-HA, BC-HA-OGP and BC-HA-OGP(10-14) wer calvarial defects. It was verified that BC-HA was
Modification of BC culture medium by adding chondroitin and hyaluronic acid (1% w/w) and *ex situ* biomimetic precipitation of CPs	BC fermentation medium was modified with cho of CP was performed from simulated body fluid
BC-HA composites synthetized by different techniques	BC-HA composites were produced by combined in the culture medium (*in situ*) and synthesis of
Sand Dollar skeleton coated by BC and CP	The skeleton of sand dollar (*Clypeaster subdepr* coated with calcium phosphates. Sand dollar sk offers a bioactive surface for cell adhesion.
BC-CPs composites with CPs from different precursors	In this report, BC-CPs composites were synth compositions. The introduction of a small numbe process of CPs.
BC/ calcium deficient HA composite	Carboxymethilcellulose (CMC) was added to th suspension of HA, before the formation of BC na cell viability.

Fig. 2 - Annual publications of BC, BC with TE applications and BC-CPs compo

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aggregation of HA and BC suspensions, introduction of the HA suspension HA in the medium of dispersed cellulose.	[44]
essus), that is formed by interconnected pores, was coated by BC and then keleton provides a suitable geometry for bone regeneration, while BC/CP	[45]
hetized by deposition of CPs previously synthetized by three different er of Mg^{2+} ions (~5% wt), according to the authors, favored the crystallization	[46]
he BC culture medium to improve its viscosity and facilitate the posterior nofibrils. HEK cells were cultivated, demonstrating its biocompatibility and	[47]

sites since 2008 to June 2018. The search was carried out in *Web of Science*^{TM}(Cont.).

Conclusions

With the growing demand for new biomaterials for bone replacement and repair, the manufacturing of BC-CPs composites has been a viable alternative once those materials are able to mimic extracellular matrix of native bone, that is primarily compound by hydroxyapatite and fibrous collagen, namely an organic-inorganic composite. CP materials are bioactive and osteoconductive and if produced with suitable geometry and topography, have the potential to be osteoinductive, becoming closer to the "gold standard" autogenous therapy. In alignment to this, some studies have been translated into efforts to enhance biomechanical performance of those composites and also the rates of cell proliferation, migration, adhesion and differentiation, by associating other materials to BC-CPs systems.

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Bacterial cellulose-based hydrogel for wound healing: characterization and *in vitro* evaluation

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ABSTRACT

Bacterial cellulose (BC) has been considered a promising biopolymer with applications in several areas of knowledge, including medicine, mainly due to its ability to assist in the treatment of dermal lesions. Many groups and companies have been making efforts to develop new BC-based materials in order to add new characteristics and therapeutic possibilities. Recently, Seven Indústria de Produtos Biotecnológicos Ltda company developed a BC-based hydrogel aiming to verify the interaction among the formulation components, its potential for wound healing and biocompatibility studies. BC-based hydrogel was characterized and compared with pristine BC film. Physicochemical characterization includes rheological measurements, thermal analyses, field emission gun - scanning electron microscopy (FE-SEM) and *in vitro* cell migration. BC-based hydrogel showed adequate interaction among the components of the formulation, which may positively influence its stability. In addition, the BC-based hydrogel accelerated the healing processes demonstrating its potential in dermal lesion treatment.

Introduction

Cellulose is the most abundant biopolymer on earth frequently obtained from plant sources. However, plant cellulose and BC show properties fairly different, which allow its applications in various fields such as physics, chemistry engineering and biological sciences. Additionally, BC production is an extremely pure process, which is entirely free of pectin, lignin and hemicelluloses, withoutcomponents from animal origin and without causing any allergic reaction leading to simpler purification process related to plant cellulose (1,2).

Currently, several microorganisms have been reported with the ability to produce BC, however Gram-negative bacteria of *Gluconacetobacter* genus have received great prominence in recent years due to their capacity to producecellulose in commercial quantities. During the biosynthesis, thesebacteria are able to synthesize cellulose in the form of membranesat the air/liquid interface of the static culture medium. These membranes present highly porous structures constituted of a random microfibrillar 3D-network of cellulose chains aligned in parallel with high permeability to fluids been favorable for adhesion and proliferation of cells (2-6).

BC membranes have shown to be a promising biomaterial for treatment of wounds healing, burns, tissue implants due to its unique properties such as high crystallinity, high mechanical strength, ultrafine fiber network structure andhigh water-uptake capability (water content > 90 %). BC provided a humid environment to the affected region promoting the exudate absorption and the wounds healing acceleration without any toxicity (1,2,4,7,8). In addition, randomly arranged cellulose nanofibers mimic some components of the extracellular matrix, such as collagen fibers, since they have similar diameter (near to 100 nm), which promotes a faster healing process (9).

BC membranes have been commercialized as an ideal wound dressing device due to its high *in vivo* biocompatibility and great efficacy when applied in cutaneous lesions promoting healing more efficiently than other products available for this purpose. In addition, BC membranes consists onphysical barriers that reduce pain and bacterial infections (6,10).

The mechanical treatment (defibrillation process) of BC membranes originates a dispersion of nanofibers

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which may to be incorporated into hydrogels, giving rise to a new therapeutic possibility for the treatment of burns, chronic ulcers, skin lesions and other lesions, protecting tissues formed around and over the wound (11). The possibility of treating deeper wounds, in which CB membranes are not capable of shaping the injured area, has been the subject of intense research by Seven Indústria de Produtos Biotecnológicos Ltda. (Brazil), since this characteristic can lead to successfully commercialization of BC-based hydrogel (e.g. Nexfill®).

Hydrogels are three-dimensional configurable polymers network with ability to absorb large amounts of water, saline and physiological solutions compared with general absorbent materials. They show excellent hydrophilic properties along with their high swelling ratio and biocompatibility, promoting their widely usage in biomedical, tissue engineering and drug delivery. Other characteristics of hydrogels are the long-term stability, facility of biochemical modification of the formed structures and the incorporation of several products inorder to combine the most important characteristics of each one (12-17).

To the best of our knowledge, few studies have investigated physicochemical characteristics and *in vitro* properties of BC-based hydrogel. Herein, we report the evaluation of the hydrogel containing BC as well as the interaction between the formulation components keeping the wound healing properties of the BC without toxicity effects.

Materials and methods

Materials

BC-based hydrogel (Nexfill® Hydrogel) was provided by Seven Indústria de Produtos Biotecnológicos Ltda. (Ibiporã, PR, Brazil) for further characterization and *in vitro* evaluation. The BC-based hydrogel (Nexfill® Hydrogel) composition was obtained according to the PI 0601330-9 A2 patent.

Rheological properties

Rheological properties of the BC-based hydrogel (Nexfill® Hydrogel) were evaluated using an Anton Paar rheometer (MCR302), equipped with two parallel-plates (PP 25) sensor with a 25 mm, the gap between plates was 1.00 mm and temperature of 32 °C. Rheo Compass™ software was used to analyze the data.

The Flow curve was analyzed with shear rate range from 0 to 100 Pas^{-1} for the ascent ramp for 120 s and from 100 to 0 Pas^{-1} for the descent ramp for 120 s and it was applied "Power Law" model, according to Equation 1:

$\tau = K \times \gamma^n \; \tau = K \times \gamma^n$ (Equation 1)

Where τ is shear stress, γ is shear rate, K is consistency index and *n* is the flow rate. In this model, $n > 1$ represents a dilatant fluid, $n < 1$ represents a pseudoplastic fluid and $n = 1$ represents a Newtonian fluid (18).

The range of frequencies used was 0.1 to 500 rad s^{-1} at 50 % strain, which proved to be in the linear viscosity range.

The storage modulus (G'), loss modulus (G") and complex viscosity (η*) were performed as a function of angular frequency range of 0.1–500 rad s^{-1}.

Thermal analysis

Lyophilized BC-based hydrogel (Nexfill® Hydrogel) was evaluated by differential scanning calorimetry (DSC) technique using a DSC1 STARe System-Mettler Toledo. The sample of ± 5 mg were submitted to heating from 25to 200 °C at 10 °C/min under nitrogen atmosphere. Thermogravimetric analysis (TGA) and differential thermogravimetric analysis (DTG) of the lyophilized samplewas performed on TA Instruments (SDT-2960) (New Castle, DE, USA). Sample (5 mg) was accurately weighed in coated alumina pan and heated from 25 to 600 °C at 10 °C/min under nitrogen atmosphere. DSC and TGA from pristine BC films were similarly obtained and compared with hydrogel results.

Morphology of BC-based hydrogel

The surface morphology of the lyophilized BC-based hydrogel (Nexfill® Hydrogel) was investigated by scanning electron microscopy FE-SEM on a JEOL microscope (model JSM-7500F, Japan). The sample was frozen at -80 °C and lyophilized for 24 h. After that, lyophilized sample was attached to slab surface with double sided adhesive tape and coated with carbon as conductive material. The sample was examined using an accelerating voltage of 2 kV. FE-SEM from pristine BC films were also obtained as described by Machado et al. 2016 and compared with hydrogel results.

Fibroblast growing model

Fibroblast culture

Fibroblast cultures internally isolated by Invitrocell, of city of Paulínia, São Paulo (Brazil) for donation of explant human cells. Cells were cultivated T-75 cm^2 flasks containing Dulbecco's modified Eagle's medium (DMEM), supplemented with 10 % fetal bovine serum (FBS), penicillin and streptomycin ((Sigma Aldrich®), USA), at 37 °C in humidified atmosphere containing 5 % CO_2. The medium was changed every dayuntil cells reach 80-90 % confluence, when fibroblasts were split with 0.05 % Trypsin/0.02 % EDTA.

Cell Migration (Wound healing assay)

The evaluation of fibroblast migration allows the evaluation of the ability of cells to repair an opening in the culture caused by injury. The test substance, BC-based hydrogel (Nexfill® Hydrogel) was evaluated at 3 different concentrations as defined in the cell viability assay (10, 100 and 1000 µg/mL). Cells were seeded at a density of 3 x 10^5 cells/well on 6-well plates. After 24 h, the cells were washed with PBS without calcium and it was created a "scratch" with a pipet tip. Cells with test substance

were cultured in DMEM containing 10 % FBS and the evaluation was performed after 6 h of incubation with the treatment. The migration process was observed after 6 h of incubation and all images of the group were obtained under inverted microscope (200x) with camera coupled by photograph (increase 3x). The quantification of cell migration was done through image analysis by ImageJ (version 1.48v, National Institutes of Health, USA) and the quantification of wound extension was analyzed in relation the size obtained in the group of basal (untreated) cells. β-estradiol (0.1 mM) was used as inhibitory control of cell migration. The results are expressed as mean ± standard error of the mean calculated in Microsoft Excel software using t student test. Significant differences between the control and treated groups are indicated by ***p<0.001, **p<0.01 and *p<0.05 (19).

Results and discussion

Physicochemical studies

Rheology properties

The study of flow properties is related to the deformation of the formulation when subjected to a shear stress, providing information on stability and consistency of the final product (20,21) BC-based hydrogel (Nexfill® Hydrogel) as depicted in Fig. 1.

According to Fig. 1, the hydrogel behaves as a non -Newtonian system, as it does not present a linear relationship between shear stress and shear rate. In agreement with the Equation 1 applied in flow curve (Fig. 1), it was observed that the value obtained by *n* (flow index) is lower than 1 (Table I) indicating that the hydrogel presented pseudoplastic behavior.

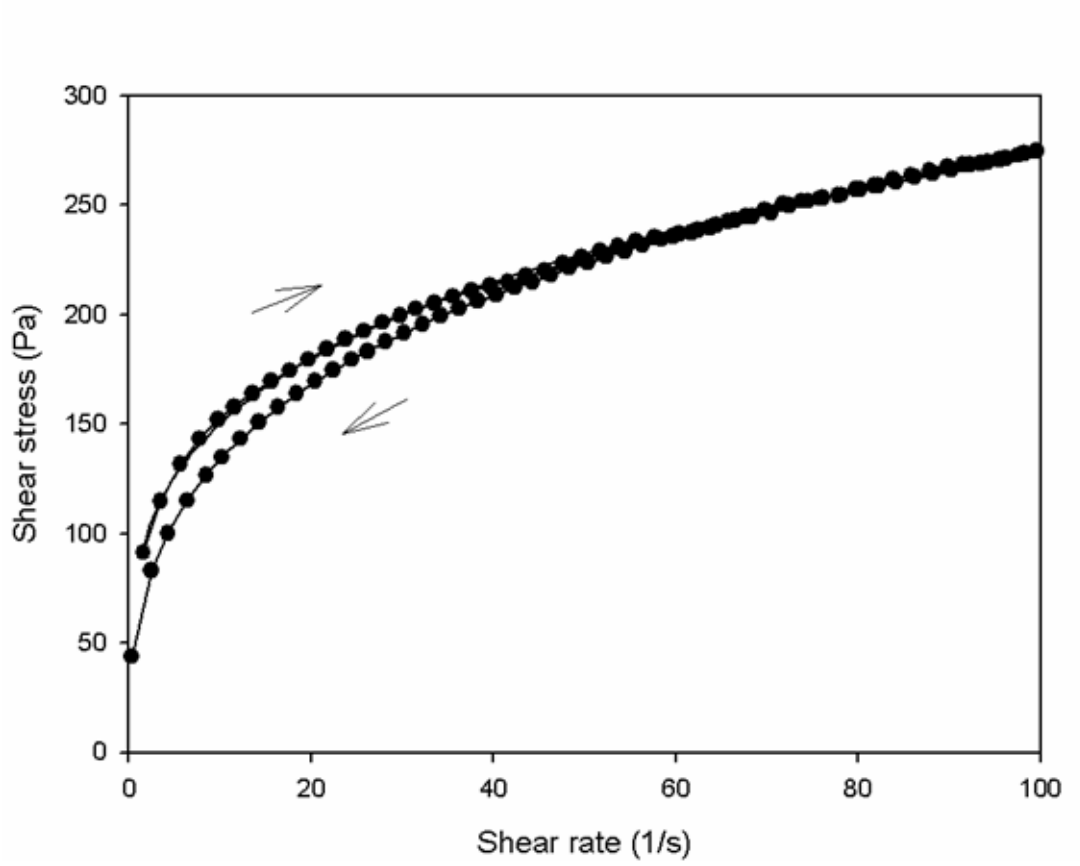

Figure 1. Tixotropic hysteresis loop of the BC-based hydrogel (Nexfill® Hydrogel).

Sample	Flow parameters		
	N	K	r^2
BC-based hydrogel (Nexfill® Hydrogel)	0.2579	83.0257	0.9987

Table I - Flow parameters of the BC-based hydrogel (Nexfill® Hydrogel) calculated by using the power law (Equation 1).

In the rheological behavior, the flow curve (Fig. 1) shows the pseudoplastic behavior that results from the alignment of the disrupted a three-dimensional network in the system in the direction of flow, providing the protective film formation characteristic that allows the skin surface to be covered, promoting a better protection (22). However, at flow curve (Fig. 1) there is hysteresis area and the hydrogel present rheological characteristics thixotropic.

Thixotropic products have the characteristic of deforming during application, becoming fluid, facilitating the scattering and recovering the initial viscosity at the time of application closure, avoiding the product to flow. Formulations with thixotropic characteristics tend to have greater self-life, because during storage it has a constant viscosity, making it difficult to separate the constituents of the formulation (23,24). Strain sweep test allows determining the amplitude in which the region of linear viscoelasticity is maintained for the sample and, through the identification of the strain values that the sample does not undergo deformation and thus other rheological tests, such as the frequency sweep.

The strain sweeps measurement was done to check for the linear-viscoelastic regime (LVE) limit and the curves are present in Fig. 2.

The hydrogel containing BC presented linear behavior until 10 %. So, a strain of 2 % was choose for the next steps, as frequency sweep.

BC-based hydrogel (Nexfill® Hydrogel) with 2 % of strain the sample did not suffer deformation and was used this value for test of frequency sweep. Frequency sweep was conducted to small amplitude oscillatory shear (SAOS) in the LVE. The ratio of the storage modulus (G ‘) and loss (G “) plotted by frequency, which provides important information about the structure of the gel (25). Important aspects of BC-based hydrogel (Nexfill® Hydrogel) structure, as well as, mechanical behavior was determinate the frequency-dependence of dynamic moduli (G’ and G”) and are present in Fig. 3.

Fig. 3 and it is observed that G’ was higher than the corresponding to the G” over the entire frequency sweep range, and the complex viscosity decreases with increasing frequency. This behavior indicates hydrogels possess a solid-like gel structure (26).

The Frequency sweep (Fig. 3) provides information about the storage modulus (G’) indicating the energy stored in the material and depends on the rearrangements that occur during the period of oscillation, which may characterize an elastic or solid character. On the other hand, the loss modulus (G”) indicates the energy dissipated or lost during the period of oscillation, which may characterize a viscous or liquid behavior. Thus, when there is a predominance of the elastic modulus (G’) on the viscous modulus (G”) it is an indication that the analyzed system is more structured and there is a strong interaction between the components (27).

Similar results were observed (28) with gelatin hydro-

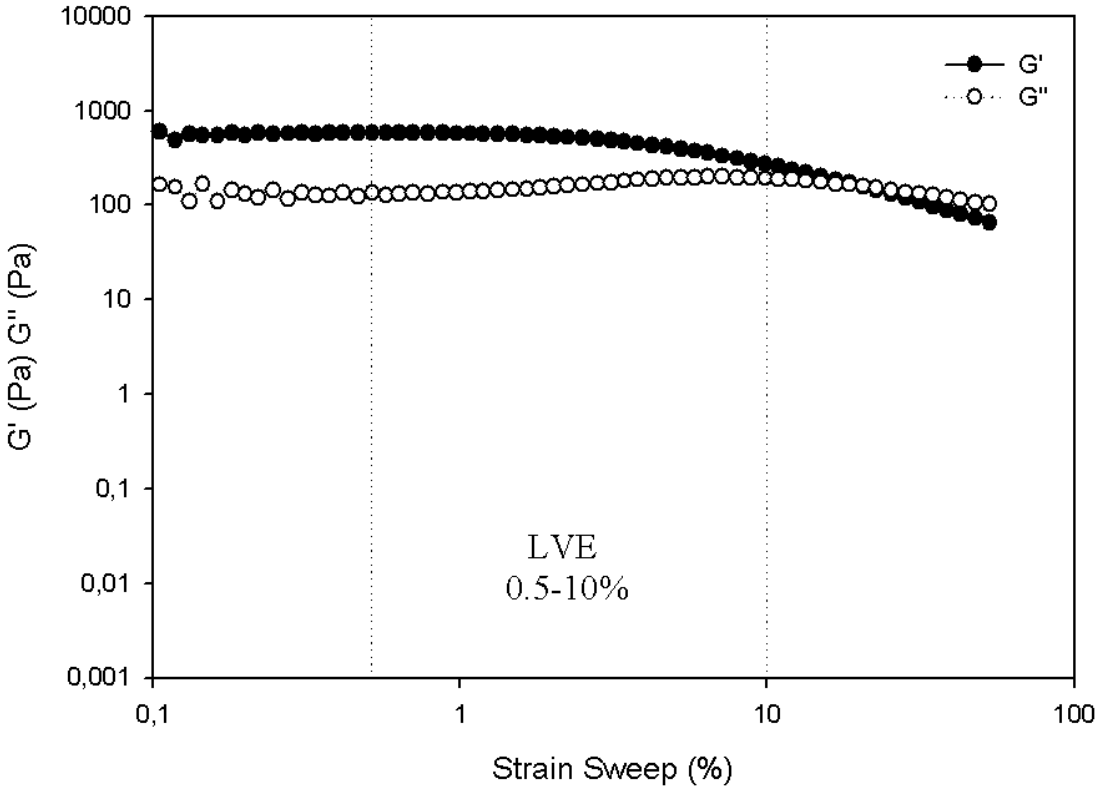

Figure 2 - Strain Sweep of the BC-based hydrogel (Nexfill® Hydrogel).

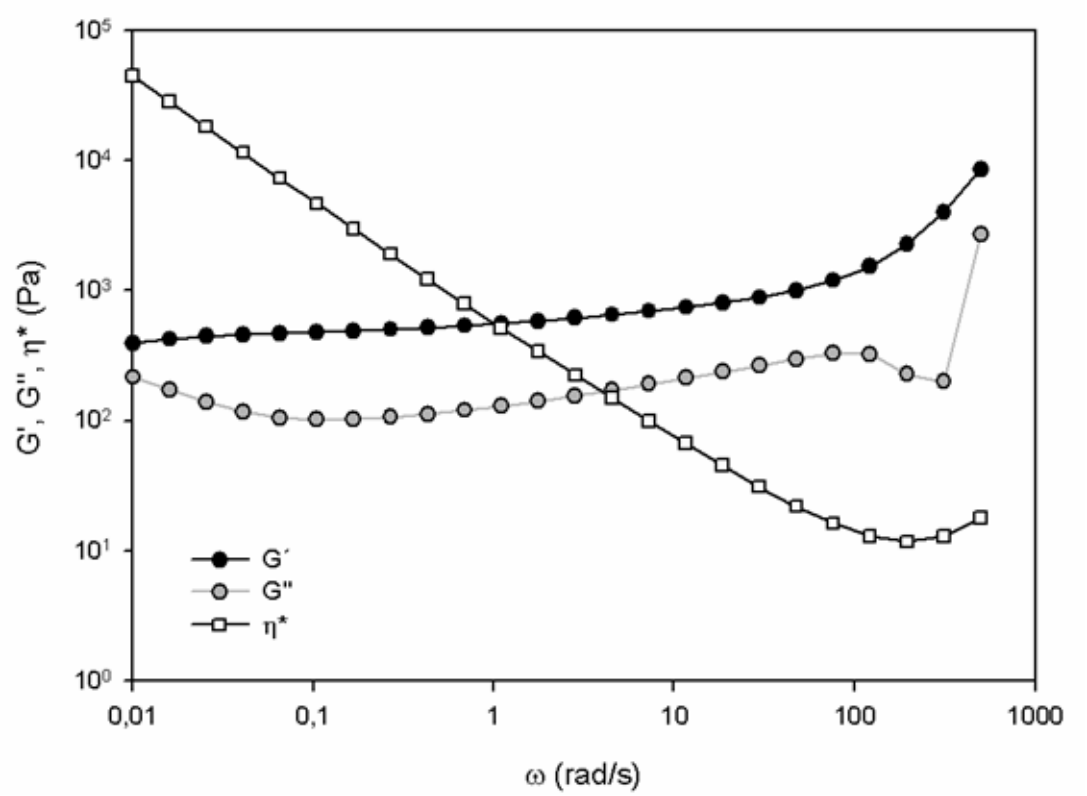

Figure 3 - Frequency Sweep of the BC-based hydrogel (Nexfill® Hydrogel).

gels reinforced with chitin of which the mechanical spectra, predominance elastic modulus (G') on the viscous modulus (G") and the G' values remained unchanged as the angular frequency (0.1–100 rad s^{-1}) indicating a strong and stiffness of the gelatin hydrogel.

Thermal behavior

Fig. 4 (A and B) shows TG/DTG results obtained from pristine BC films and BC-based hydrogel (Nexfill® Hydrogel), respectively.

For pristine BC films (Fig. 4A), two characteristic events were detected. First, the mass loss from 25 to 100 °C (3.51 %) was assigned to the water molecules. The second and main mass loss (75.46 %) starting from 250 to 450 °C (T_{onset} = 372 °C) was ascribed to the BC degradation process such as depolymerisation, dehydration and decomposition of glucose units (1,29). Four main mass loss events were observed for BC-based hydrogel (Fig. 3B). The first one, starting from low temperature to 110 °C (T_{onset} = 55 °C, 10.19 % of mass loss) corresponds to the solvents and water molecules (dehydration processes of the hydrogel) (1,29). The thermal degradation of the sample occurs in three subsequent steps (T_{onset} = 192, 322 and 377 °C). First step occurs from 110 to 250 °C (38.93 % of mass loss) attributed to the humectant compounds. The second (T_{onset} = 322 °C, 21.29 % of mass loss) and third (T_{onset} = 377 °C, 9.52 % of mass loss) steps were assigned to the degradation processes of BC and other polymers in

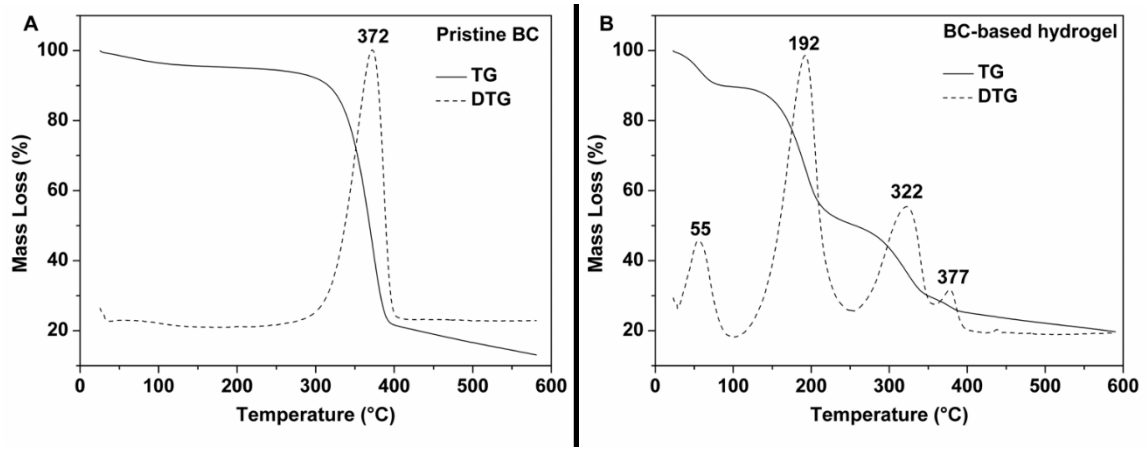

Figure 4 - Thermogravimetric (solid lines) and differential thermogravimetric (dashed lines) analysis of (A) pristine BC film and (B) BC-based hydrogel (Nexfill® Hydrogel).

agreement with pristine BC films (Fig. 4A) (1,29). Additionally, a residue of 19.70 % was detected for BC-based hydrogel (Nexfill® Hydrogel) and ascribed to inorganic salts and carbonaceous materials (carbon and carbon monoxide) (1,30).

The DSC curve of BC-based hydrogel (Fig. 5B) results showing an endothermic peak starting from room temperature to 110 °C as aforementioned in Fig. 4B, this event was assigned to solvents and water loss. It is worth noting that the hydrogel showed an endothermic event more pronounced and at similar temperature relating to pristine BC films (Fig. 5A). Although it was expected water loss from BC-based hydrogel (Nexfill® Hydrogel) at lower temperatures than for pristine BC film, the hydrogel shows similar behaviour to pristine BC film. These results suggest a strong interaction of the components seen in the hydrogel through the rheological measures to oppose to water loss.

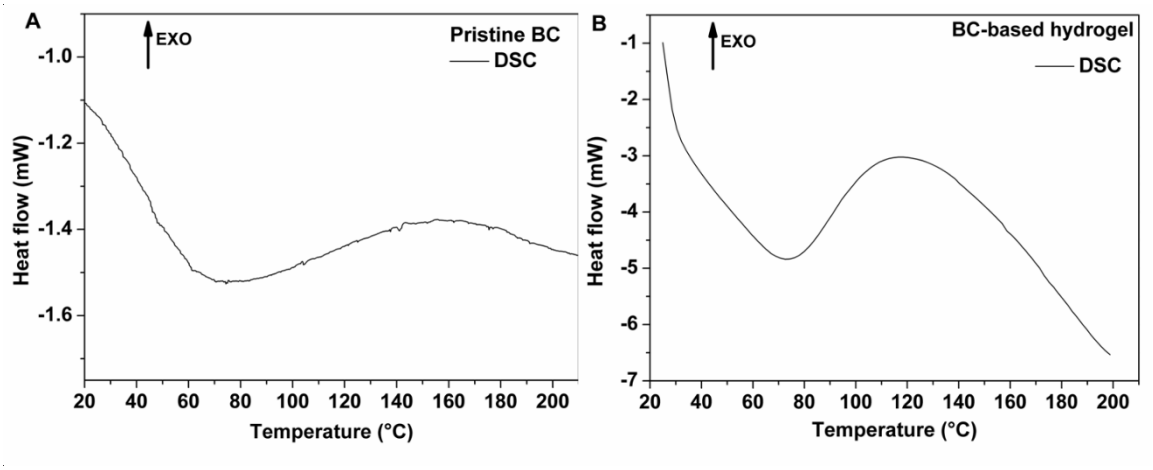

Figure 5 -. Differential scanning calorimetry curves of (A) pristine BC film and (B) BC-based hydrogel (Nexfill® Hydrogel).

Morphology analysis

The morphology of dried pristine BC film and lyophilized BC-based hydrogel (Nexfill® Hydrogel) were investigated by FE-SEM as shown in Fig. 6.

Fig. 6A displays pristine BC film composed by three-dimensional network porous structure containing randomly arranged cellulose nanofibers (31). Fig. 6 (B-E) exhibit BC-based hydrogel (Nexfill® Hydrogel) with BC nanofibers clearly and well dispersed on its surface. These results suggest that the hydrogel could keep the characteristic properties of pristine BC films in its composition (8,32).

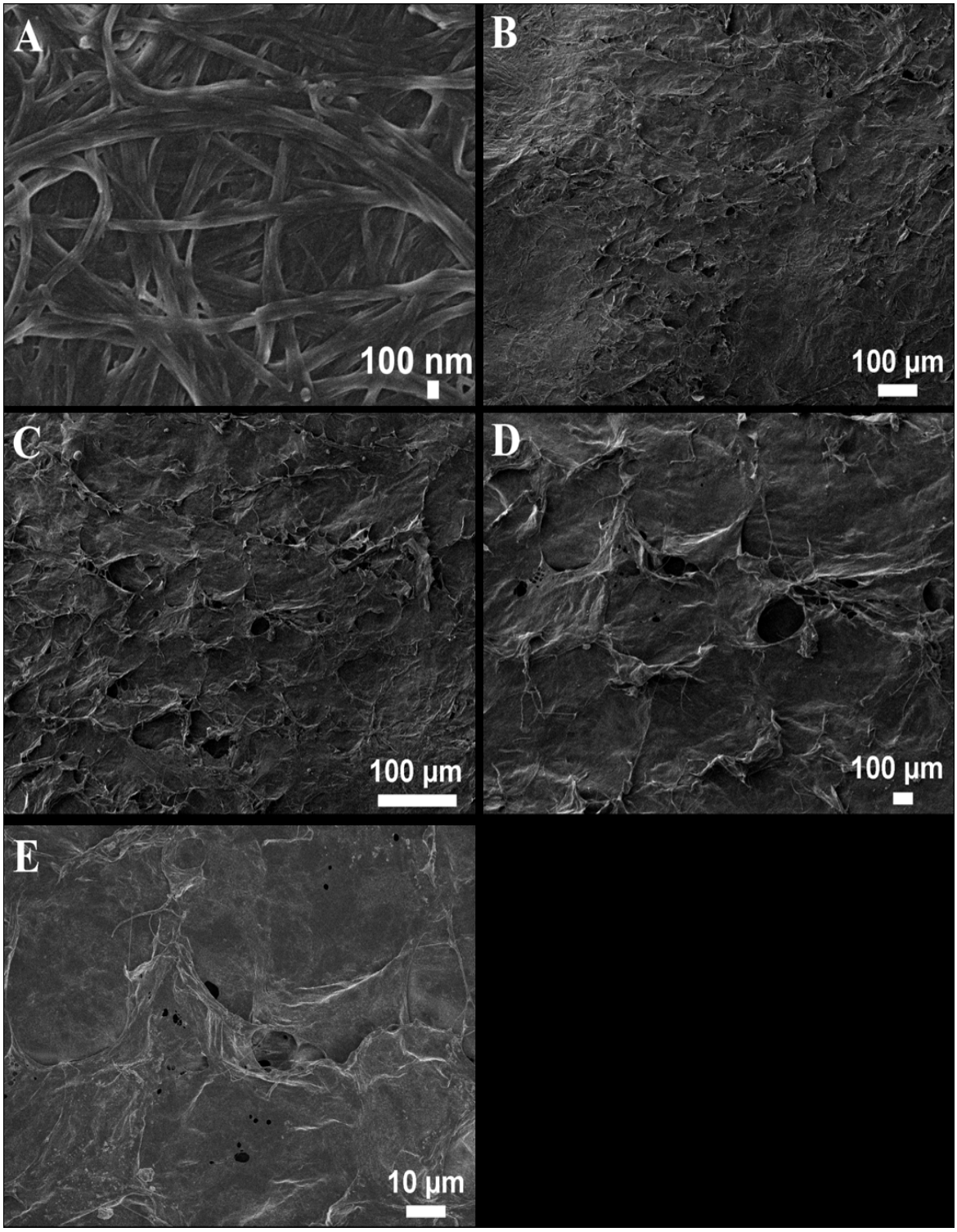

Figure 6 - FE-SEM of the pristine BC film (A), BC-based hydrogel (Nexfill® Hydrogel) (B) 100x, (C) 200x, (D) 500x and (E) 1000x.

In vitro studies

The fibroblast migration ability was also evaluated after opening a ''scratch'' in the middle of the semi-confluent culture of fibroblasts after 6 h of treatment with BC-based hydrogel (Fig. 7).

Fig. 7 illustrates healing activity of different concentrations (10, 100 and 1000mg/mL) of the BC-based hydrogel (Nexfill® Hydrogel) related to the basal control (untreated cells). The reference substance β-estradiol promotes delay in wound closure presents a statistically significant difference when compared to baseline control ($p<0.001$). BC-based hydrogel (Nexfill® Hydrogel) showed the highest significant when compared with basal control ($p<0.001$) in 1000 mg/mL, demonstrating that in this concentration the highest fibroblast migration toward the scratched area almost closing the wound while at 100 and 10 μg/mL a delay in fibroblast migration was observed relating to the basal control ($p<0.01$ and $p<0.05$, respectively).

Similar results were also observed (33), which evaluated chemically modified BC membranes and demonstrated good *in vitro* compatibility with fibroblasts, once the membrane helped in the healing process.

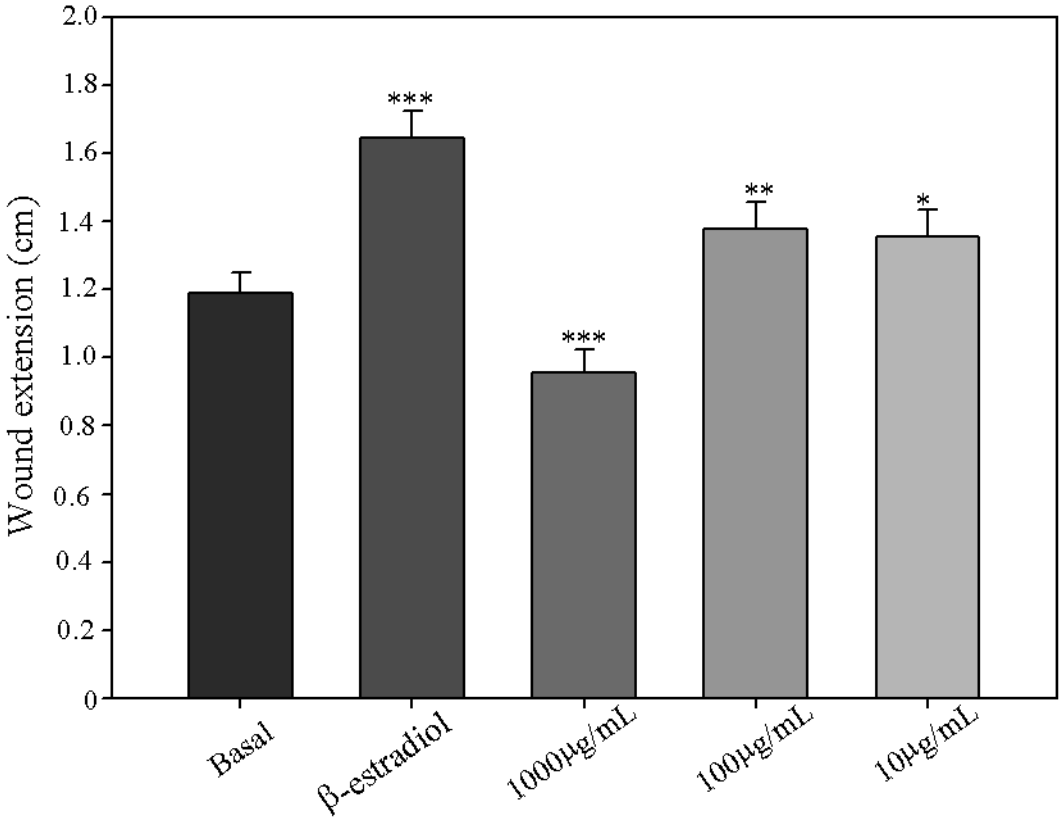

Figure 7 - Evaluation of the fibroblast migration capacity of the BC-based hydrogel (Nexfill® Hydrogel). The plot shows mean values ± Standard error of the mean obtained for each treatment. The values differ of the basal control at *** for when $p<0.001$, ** for when $p<0.01$ and * for when $p<0.05$ in the student t statistical test.

Conclusions

BC-based hydrogel (Nexfill® Hydrogel) were developed as a potential strategy for the treatment of chronic wounds in which membrane occlusion is not adequate, or even for those cases where the depth of the lesion promoted by tissue loss hinders the adaptation of the membranes in the wound bed. As expected, the BC nanofibers present in the hydrogel were responsible for the building of a strong and structured network which should lead to a high interaction pattern with the biological interfaces but allowing its adequate and comfortable spreadability on the wounds. The set of data suggest that the BC-based hydrogel consists on a suitable formulation for wound repair since fibroblasts represent the first defense line against injuries and the increase of fibroblast proliferation in wound bed is fundamental for lesion repair.

Conflict of interest

There are no conflicts to declare.

Acknowledgements

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Bacterial cellulose-based biomaterials on third-degree burns in rats

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ABSTRACT

Burns are cutaneous lesions that present high rate of morbidity and mortality worldwide. In order to innovate the treatment strategies currently applied new biomaterials are being investigated. The aim of the present study was to evaluate the action of bacterial cellulose in both membrane and gel form, in the treatment of third degree burns in rats. For this, 24 Wistar rats were used, divided into three distinct groups. The lesion was performed with the aid of a soldering iron heated at 150 °C pressed on the back of the animal for 10 seconds. Treatment was performed immediately after wound induction, and skin samples were collected on the tenth day post-injury. Statistical analysis was performed using a significance level of 5% ($p \leq 0.05$). The histological results show differences in the healing process presented by each group. The group that received bacterial cellulose in the membrane format presented the best results, such as discrete inflammatory infiltrate and better morphological quality of the tissue, characterizing an advanced stage of the healing process, also proven in the collagen quantitative analysis. On the other hand, the group that received the cellulose gel showed characteristics of an inflammatory phase with the presence of evident ulcerations, which corresponds to a delay in the healing process even when compared to CG alone. Thus, it was concluded that before the biomaterials tested cellulose membrane in the format presented more favorable results both in terms of environmental protection as a contribution to an adequate tissue recovery.

Introduction

Burns are considered severe injuries occurring due to exposure of human skin to chemical, physical or biological agents, and the severity related to the extent and depth of the damaged area (Pessolato et al, 2011; Knabl, et al., 1999). Most cases seen in the public health system are serious injuries of difficult clinical intervention, and because of this its morbidity and mortality are high.

The healing process is complex and requires the collaboration of different cell types (Sun et al, 2011). Still being didactically divided into three overlapping phases, called inflammation, proliferation and remodeling (Sun et al, 2011;. Scwacha et al., 2010). However, in deep and/or extensive lesions tissue reestablishment becomes a challenge, and thus, the end result of healing can be impaired, altering local mobility and innervation and presenting significant tissue fibrosis (Pantoja et al., 2006).

Because of this, new treatment approaches have been proposed in an attempt to meet the local needs so that the tissue healing process evolves quickly and effectively. (Baxter et al., 2012). Biomaterials, natural and synthetic, aim to improve the functionality of organs or tissues (Labus et al., 2012, Maia et al., 2010), and are being extensively investigated for biomedical applications (Abeer, Amin, Martin et al., 2014; Czaja et al. 2007).

Bacterial cellulose is a biopolymer formed by an extracellular polysaccharide produced in a static culture medium by several types of bacteria (Avila et al., 2014; Abeer, Amin, Martin et al., 2014). Its characteristics such as biocompatibility, purity, crystallinity and stability confer ideal conditions for biomedical applications, including natural curatives or skin substitutes (Chen, 2009). In

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addition, deposition of the nanofibers in a 3 D structure results in a broadly nanoporous surface, which facilitates selective permeability, and protects the wound environment from harmful agents from the external environment. Other peculiar properties such as hydrophilicity, resistance and adequate adhesion on irregular surfaces of the body, make this biomaterial valuable, given the possibilities of applications that encompass areas such as science, medicine and biotechnology (Cheng et al., 2014). They also promise to significantly innovate the area of tissue engineering, as they demonstrate resistance and adequate adhesion which allows their application in chronic wounds such as ulcers and severe burns. (Almeida, et al., 2014; Saska, 2011).

In view of the abundance of characteristics presented, in addition to its macromolecular structure, this type of bacterial cellulose is also being directed to the manufacture of topical products like ointments and / or gels in an attempt to facilitate the application in extensive wounds. It is important to highlight that this new method of using bacterial cellulose is innovative, since the literature does not present expressive and scientific methodological evidences that already prove its real benefits.

Therefore, the objective of this work was to evaluate the effects of bacterial cellulose in both membrane and gel form in the treatment of third degree burns in rats.

Material and methods

For this study, 24 male Wistar rats (12 weeks old, 280 ± g) were used. The animals were randomly distributed in three experimental groups, with 8 animals each, control group (CG), where the animals were submitted to the burn, without any treatment; membrane group (MG), submitted to burn and treated with bacterial cellulose membrane; gel group (GelG), burned and treated with bacterial cellulose gel. All animals were kept in individual cages, temperature controlled (19-23 ° C), dark light cycle (12-12 hours) and with free access to food and water. All the study was carried out according to the manual of care and use of animals in the laboratory and approved by the Committee of Ethics in Animal Experimentation of the Federal University of São Carlos, 022/2013.

Experimental procedure

For the burn procedure, the animals were anesthetized with ketamine (95 mg / kg) and Xylazine (12 mg/kg) intraperitoneally and then trichotomized. The burn was performed on the back of each animal with a 1 cm2 aluminum plate coupled to a soldering iron (Kimura et al., 2006; Ko et al., Busuioc et al., 2013) with a temperature of 150°C, controlled by a thermostat and pressed on the animal's skin for 10 seconds (Ko et al., 2013; Campelo, et al., 2011). Immediately after injury the animals received 6.2 mg/kg^{-1} of dipyrone sodium, and then the treatment proposed for each group. The application of bacterial cellulose in membrane form was performed only once and maintained throughout the experimental period, the gel cellulose was applied on intercalated days, completing at the end of the treatment 5 applications. Ten days after the induction of the lesion, the tissue samples were collected and sent for the analysis.

Bacterial Cellulose

Both biomaterials were manufactured and assigned to the study by DMC Equipamentos - Ltda., São Carlos/SP, Brazil. They were obtained by culturing strains of bacteria of the genus Acetobacterxilynum in appropriate media of cultures that favor the formation of cellulose nanofibres, forming as final product a highly hydrated membrane. After obtaining the pure membrane, the membranes were treated and cleared. To obtain its increased lidocaine variable, this membrane, still in its wet state, underwent a deposition process, where they were subjected to a controlled spray of 20 ml of aqueous solution containing 4% lidocaine. At the end of the procedure the membranes were kept in an oven at 80°C for the drying process.

For the gel formulation, the same procedures used in the production of Biocel dressings already registered by the company DMC Equipamentos Ltda, São Carlos/SP (Anvisa registry - 80030810109) were used, plus gel composition, 50% bacterial gel cellulose gel, 0.15% nipagin (antifungal), 12% CRS crodabase, 3% ginger and 30.85% purified water.

Histopathological Analysis

After the experimental period, the total area of the burn was removed for the analysis. The samples were fixed in 10% buffered formalin (Merck, Darmstadt, Germany), embedded in paraffin and cut into cross sections with a standard thickness of 5 μm. Three cuts of each sample were then made, which were subsequently stained with hematoxylin and eosin (HE, Merck) and analyzed. The histological evaluation was performed by a pathologist blind to the treatment, on a light microscope (ZEissAxioshop, Carl Zeiss, Rio de Janeiro Brazil, with a 40x objective). The following parameters were evaluated: presence of fibrosis, ulcerations and inflammatory infiltrate (Brassolatti et al., 2016).

Quantitative analysis of blood vessels

For the quantitative analysis of blood vessels, three distinct fields with a 10x objective were captured from the dermis region of each histological section with the aid of a Motican 5.0 imaging program. The fields were divided into C1 corresponding to the central region of the lesion, C2 corresponding to the left border of the lesion and C3 corresponding to the right border of the lesion. From this, the vessels present in each field were counted with the help of the Image J program. Subsequently, an average number of vessels per animal was determined, and then the mean of each experimental group was calculated. The entire calculation was considered by statistical analysis (Nunez et al., 2013, Bossini et al., 2009).

Morphometry of collagen fibers

Histological sections stained with the picrosiriri red method were analyzed in a polarized light microscope to evaluate and quantify deposition of collagen fibers in the dermis region. The collagen analysis is based on its birefringent properties, where type I collagen fibers appear in orange or red coloration (Gonçalves et al., 2013; Dantas et al., 2011). For this, three consecutive fields located in the central region of each sample were photographed using a camera coupled to a polarized light microscope at a magnification of 200x (Colombo et al., 2013). For the calculation, the Image J program was used, which gives the percentage of collagen fibers per area in pixels, and then the mean of each group was calculated (Nunez et al., 2013). All analyzes were performed in a blinded study by an experienced pathologist (Pessolato et al., 2011).

Statistical Analysis

For all the analyzes of comparison between the groups studied, one-way analysis of variance was used, complemented later with the Tukey test. For the statistical analysis, the PRISMA software version 5.0 (Software-Soft Inc system) was used, where values of $p < 0.05$ were considered significant.

Results

Histopathological analysis

Histopathological analyzes revealed differences among all the groups evaluated. The bacterial cellulose membrane proved to be effective in protecting and assisting the healing process, demonstrating a morphological pattern compatible with a more advanced stage of repair when compared to the control group and the gel membrane group. In the MG group it was possible to observe characteristics of complete tissue repair because of the formation of the epithelium, presence of the skin attachments, organization of the collagen fibers, discrete inflammatory infiltrate, discrete granulation tissue and absence of ulceration and fibrosis. Differently the CG presented thick epidermis and disorganized tissue with absence of skin attachments, moderate inflammatory infiltrate, moderate granulation tissue and evident characteristics of tissue fibrosis. Similarly, GelG also presented moderate inflammatory infiltrate but with a slight presence of indicative of tissue fibrosis. In addition, this group differed from the other two evaluated CG and MG due to the presence of ulceration due to the discontinuity or non-reconstitution of epidermal tissue (Fig. 1).

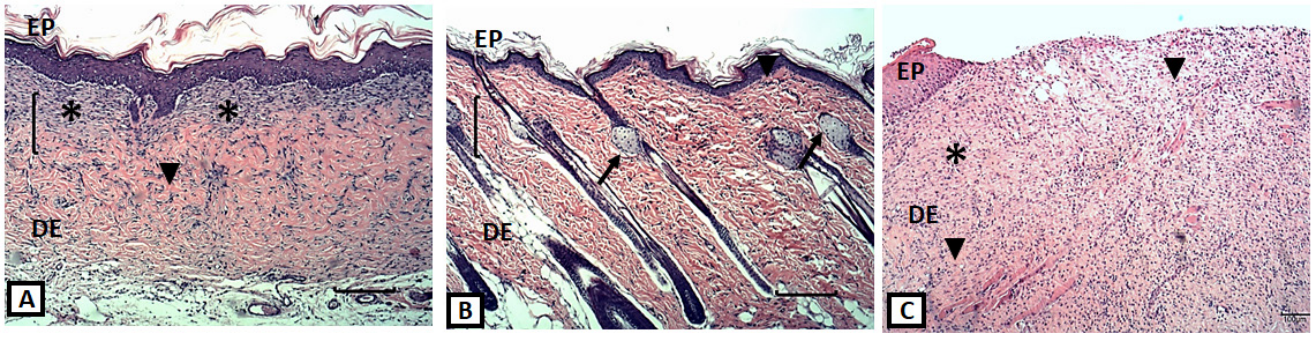

Figure 1- Representative photomicrographs of experimental groups stained with hematoxylin and eosin. (EP) epidermis, (DE) dermis, (*) fibrosis, (black arrow) skin attachments, (▼) inflammatory infiltrate. A - control group (CG) representing the skin only with the lesion, B - bacterial cellulose membrane group (MG), C – bacterial celulose gel group (GelG).

Morphometry of blood vessels

Blood vessel counts were predominantly performed on the dermis layer. A statistically significant difference was observed in the comparison of the MG group with CG and GelG, and MG had the highest number of blood vessels. In the comparison of Cg with GelG, a statistically significant difference was also found, in which GelG demonstrated the lowest amount of blood vessels. This same observation was found when comparing the MG and GelG groups (Fig. 2).

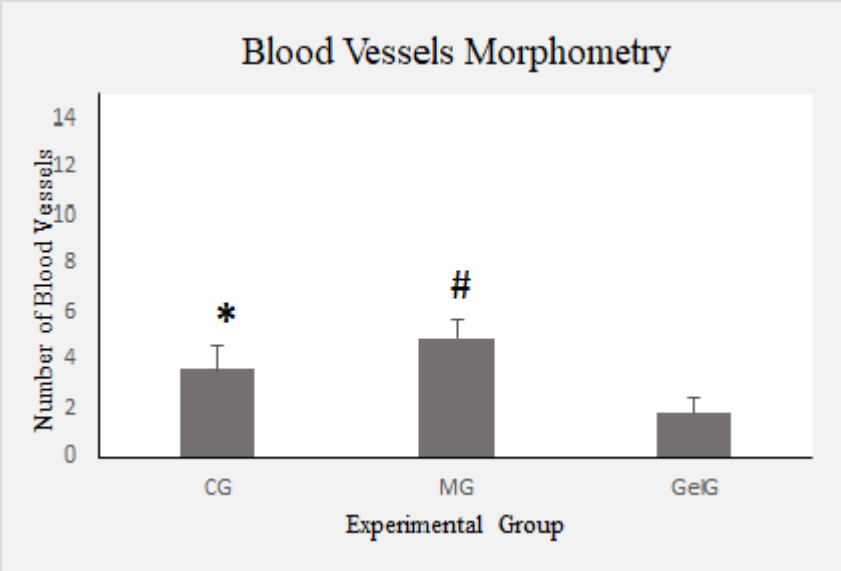

Figure 2 - Number of blood vessels. CG control group; MG bacterial cellulose membrane group and GelG bacterial celulose gel group.

Birefringence of collagen fibers

Figure 3 shows the percentage of collagen fibers evaluated in each experimental group. The MG presented a statistically significant difference in relation to the other two groups (CG and GelG), demonstrating a greater amount of collagen fibers in the dermis region. In the comparison of CG and GelG groups, no significant statistical difference was observed.

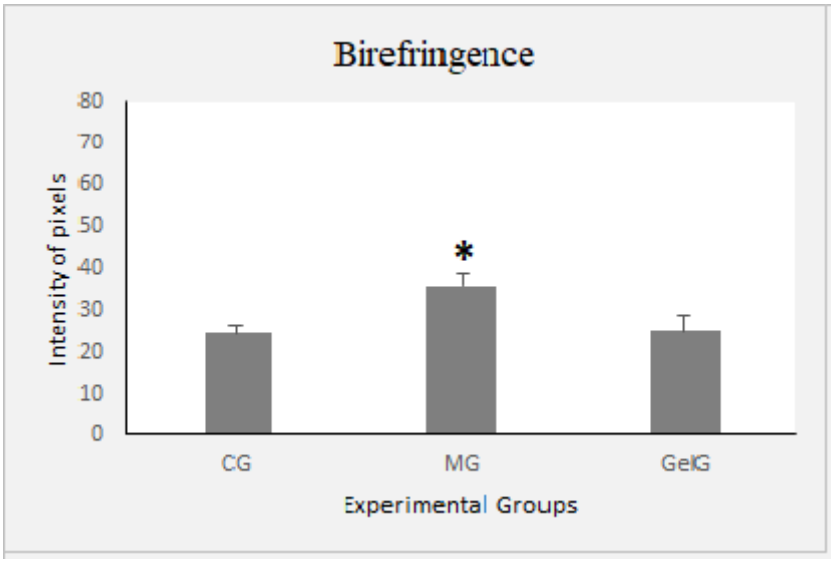

Figure 3 - Percentage of collagen fibers. CG control group; MG bacterial cellulose membrane group and GelG bacterial celulose gel group.

Discussion

The search for new biomaterials able to innovate the areas of regenerative medicine and tissue engineering is growing these days. This study aimed to investigate the bacterial cellulose membranes contribution both in format as gel in third-degree burns. The properties of biomaterials bacterial cellulose based are found in the literature (Almeida et al, 2014; Fu et al, 2013; Abeer et al, 2013, Czaja et al., 2007), but the information regarding a contribution in third-degree burns are still scarce.

The skin tissue has a marked regenerative capacity that is closely related to the kind of evolution of healing (Busuioc et al., 2013) because complications in one of the phases as bacterial infections or even molecular and genetic disorders can disrupt both the aesthetic result of wound healing intrinsic functionality. Biological dressings, in turn, appear to act as functional protective barriers, that is, they promote an effective barrier against microorganisms, but it also helps the injured environment through its selective permeability and its functionalized 3D structure which contributes to the processes of migration and cell proliferation.

Fu et al., 2012, compared the effects of different types of treatments on full-thickness wounds on the back of mice. The results demonstrated that bacterial cellulose-based biomaterials presented advantages during healing, with a decrease in the inflammatory response when compared to the groups treated with conventional grafts and dressings. In addition, they report that the macromolecular structure of the biomaterial acted satisfactorily in protecting the wound bed preventing possible infections. Brassolatti et al., 2018 evaluated the action of two distinct types of bacterial cellulose membranes and observed that the use of biological dressings in third degree burns in rats prevented infections and presented a significant evolution in the healing process when compared to the control.

Histologically, our results regarding the use of bacterial cellulose in the form of a membrane corroborate with the previous findings described, since we observed that the tissue morphological structure of this group presented better quality when compared to the others. It should be noted that a positive result was also found in relation to the inflammatory process of the tissue, which in this group was presented in a light form, evidencing that the evolution of the healing process evolved quickly and effectively. There is evidence that the outcome of the inflammatory phase is closely related to the formation of bedsores and fibrosis (Lee et al., 2003; This fact is interesting to discuss because in the control group as well as in the group of bacterial cellulose gel a moderate infiltrate was observed, and in the group that received the gel there was a significant ulceration of the epidermis, characterizing a significant healing delay for the period evaluated.

The synthesis of collagen is a key process of being evaluated in the transition from the inflammatory to the proliferative phase, because when its levels are high they are harmful and indicate the formation of fibrosis due to excessive formation of extracellular matrix (Pessolato et al., 2011). Brassolatti et al., 2018 evaluated the percentage of collagen fibers and did not find significant differences between the groups treated with the membranes and the control. In contrast, we observed in our study that the group that received cellulose in the form of membrane presented a percentage of fibers more pronounced than the other two groups evaluated.

From the results found in this work, important observations should be highlighted regarding the mode of use of bacterial cellulose. The cellulose gel did not present satisfactory results, on the contrary, it seems to have delayed the evolution of cicatrization. This may be related to a possible accumulation of the product in the wound environment due to the numerous applications, or also because the structure of the gel necessitates the association of other chemical components for its stability. However, when bacterial cellulose was used in its pure form the membrane structure favored the healing process and presented a satisfactory tissue morphological quality by the type of lesion.

Thus, it is possible to conclude that the bacterial cellulose used in the membrane format presents favorable indications to be used as biological dressings in third degree burn frames, since they provide an adequate protection while favoring the process of cell proliferation. In relation to its gel structure, future studies are required with other formulations or even reduced application numbers in order for the evaluation to become more accurate.

Acknowledgments

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Incorporation of micro/nanoparticles of Polycaprolactone with essential oil of Cymbopogon nardus in bacterial cellulose

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ABSTRACT

Incorporation studies of particles in different substrates with herbal assets growing. The objective of this work was the preparation and characterization of micro/nanoparticles containing *Cymbopogon nardus* essential oil; and the incorporation of them on bacterial cellulose. For the development of the membranes was used the static culture medium and for the preparation of micro/nanoparticles was used the nanoprecipitation methodology. The incorporation of micro/nanoparticles was performed on samples of bacterial cellulose in wet and dry form. For the characterization of micro/nanoparticles were carried out analysis of SEM, zeta potential and particle size. For the verification of the incorporation of particulate matter in cellulose, analyses were conducted of SEM and FTIR. The results showed that it is possible the production and incorporation of micro/nanoparticles containing essential oil in bacterial cellulose membranes in wet form with ethanol.

Introduction

The essential oil of *Cymbopogon nardus (C. nardus)*, popularly known as citronella, can be used as insect repellent, insecticide and as, for example, larvicidal for *Aedes aegypti*[1-3]. It is also used to calm itching, muscle aches, rheumatic aches, headaches and as antiperspirant. The forms of use may be for massage, compress, bath, cosmetic care, inhalation, dissemination, on a neutral tablet or in food, and may be used for other various purposes[2].

The main chemical components of the essential oil of *C. nardus* are citronellal, geraniol and citronellol. These components have anti-inflammatory, sedative and antiviral properties. Citronella essential oil can contain different levels of the components mentioned by crop factors and planting[3,4]. On the other hand, essential oils are sensitive to the effects of light, humidity and high temperatures, in addition to the volatility. For these reasons, encapsulation is an important method for protect the active ingredients[5]. Thus, the objective of this work was the preparation and characterization of micro/nanoparticles containing *Cymbopogon nardus* essential oil and the incorporation of these particles on bacterial cellulose membranes with the intent to facilitate the dissemination of mentioned therapeutics characteristics.

Materials and methods

Preparation of micro-and nanoparticles containing citronella essential oil

Micro and nanoparticles were prepared in triplicate with 40% essential oil of *C. nardus* (WNF), and were kept under exhaustion during 4:00 with constant magnetic stirring for evaporation of acetone (Quimis PA), which was used as organic phase in the nanoprecipitation method. The organic phase was obtained by dissolution of 0,115g of polycaprolactone (PCL, Sigma-Aldrich, 45,000 Mw g/mol), 0,0546g of Span® 80 surfactant (Sigma-Aldrich, Mw: 428,62 g/mol) and 0,020g of citronela oil in 30 mL of acetone by magnetic stirring under temperature of about 30°C. In turn, the aqueous phase was prepared with approximately 50 mL of distilled water and 0.08g surfactant Tween ® 80 (Sigma-Aldrich, Average Micellar Weight 79.000) also by magnetic stirring under approximately 30°C. After the two solutions (organic and aqueous phase) reached the same temperature of about 30°C, the organic phase was added drop by drop with Pasteur pipette to the aqueous phase under agitation provided by ultra-turrax (20,500 rpm). At the finish of the addition of organic phase on the aqueous phase, the newly formed dispersion was lead to the acetone evaporation during two different

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times under magnetic stirring, 4 and 24 hours. Then, it was stored in amber bottles and stored away from light, at ambient temperature of about 25°C.

Incorporation of particles containing Citronella essential oil in the membranes of bacterial cellulose

The incorporation of micro/nanoparticles was performed in triplicate for each type of in bacterial cellulose (BC), dry and wet form. BC was prepared according to the method described recently[6]. The samples of BC, receipt the amount of dispersion of micro/nanoparticle containing citronella oil and impregnation aid agent shown in Table 1.

The dry BC was obtained starting from the wet BC after this being dried for 48h at 25°C. Each formulation shown in Table 1 was prepared with 3.0 x 3.0 cm samples of BC. The Petry dishes containing the BC membranes impregnated with 3 mL of micro/nanoparticles dispersion with or without impregnation aid agent were left for 72 hours in ambient temperature until complete drying by natural evaporation of water coming from the dispersions.

The zeta potential (ZP) was observed by Phase Analysis Light Sctattering (PALS). The particle size was observed by Dynamic Light Scattering (DLS). Polydispersion (PDI) was a consequence of observation of DLS. These tree properties were measured in a NanoBrook equipment, model 90 Plus/Pals. The analyses were performed in triplicate at 25°C in polystyrene cuvettes with 1 cm of optical path and volume of 4.5 mL. The light scattering was observed with an angle of 90°. As the analyses were performed in triplicate, the results are expressed as simple average and the standard deviation. The standard deviation was calculated by standard procedure.

Scanning electron microscopy (SEM) images were obtained in a Field Emission Scanning Electron Microscope JEOL JSM-6510L. Samples were coated with tick gold layer following the standard procedure.

The infrared spectra were obtained in a Perkin Elmer

Acronym	Formulation	Incorporation
BCD-3	1	Dry bacterial cellulose with 3 mL dispersion
BCM-3	2	Moist bacterial cellulose with 3 mL dispersion
BCD-33	3	Dry bacterial cellulose with 3 mL dispersion + 3 mL of ethyl alcohol
BCM-33	4	Moist bacterial cellulose with 3 mL dispersion + 3 mL of ethyl alcohol
BCD-12	5	Dry bacterial cellulose with 1 mL dispersion + 2 mL of purified water
BCM-12	6	Moist bacterial cellulose with 1 mL dispersion +2 mL of purified water

Table 1 - Description of the content of each sample of BC with incorporation of micro/nanoparticles.

Spectrum Two Spectrometer, with Universal ATR accessory (UATR), in the range of 4000 to 450 cm^{-1} with resolution of 32 cm^{-1} and 4 scans per spectrum.

Results and Discussion

Characterization of micro/nanoparticles

In order to check the morphology of the micro/nanoparticles prepared in this work, micrographs from dry material were obtained. Figure 1 presents micrographs of three dispersions of micro/nanoparticles with citronella essential oil formulations (a, b and c), with 4 hours of solvent evaporation, which shows high similarities. Figure 1 denotes the formation of micro/nanoparticles in all three formulations, most of them exhibiting spherical and uniform shapes. In these micrographs, also could be seen that the size of the particles features inhomogeneity points.

This inhomogeneity could be related to the organic phase droplet size dispensed into the aqueous phase[7], which was controlled manually. Thus, these differences in the size (nano and micro) of particles were expected. It is worth to note that the solvent evaporation time apparently does not affect significantly the shape of the particles, as can be seen in the Figure 2.

Figure 2 presents micrographs of three dispersions of micro/nanoparticles with citronella essential oil formulations (d, e and f), with 24 hours of solvent evaporation which, also, shows high similarities.

The particle size may vary depending on the amount of oil in relation to the polymer and, in some cases, the same can also occur with the rise of oil/polymer ratio

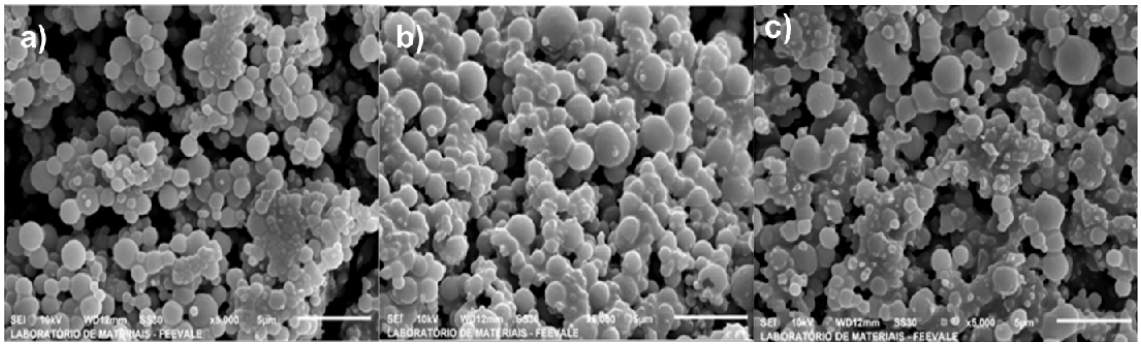

Figure 1 - Micrographs of micro/nanoparticles formulations (a), (b) and (c), with 24 hours of solvent evaporation (5000 x).

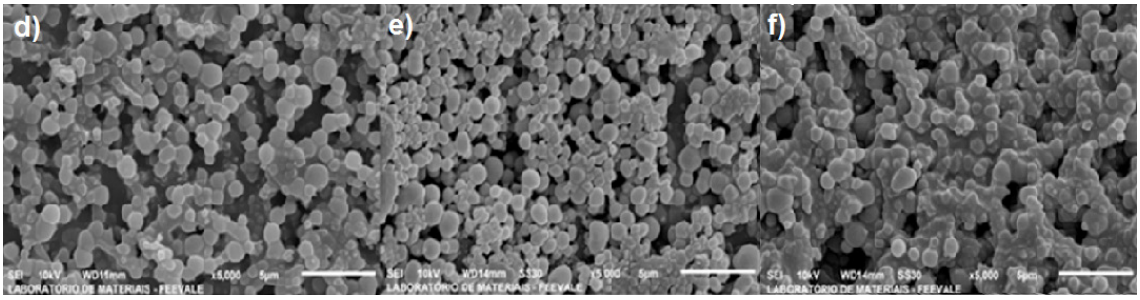

Figure 2 - Micrographs of micro/nanoparticles formulations (d), (e) and (f), with 24 hours of solvent evaporation (5000 x).

in the organic phase. This correlation could be a factor responsible to increase the resistance to diffusion of the organic phase into the aqueous phase, allowing a greater association of the active/oil in the nanoparticles[8,9], but the huge difference, nano and micro, could not be attibuted to a factor like this. On the other side, it is well known that the measures of size performed by SEM morphology analysis always exhibit sizes greater than those measured by DLS[10]. In this sense, the difference in particle size by DLS and by SEM can be related to the fact that the preparation of the latter requires a drying procedure and sample preparation prior to analysis, which are conducted, as well as own analysis, under high vacuum. This vacuum exposure during the preparation and analysis should be responsible for enforcing essential oil volatilization and consequently, dilatation of the particles[11].

Thus the quantitative analises of particle size by DLS should provides better results. The particle size and standard deviation of each of the six formulation, measured by DLS, are presented in Figure 3.

Figure 3 shows the particles sizes of the formulations *a* to *f*. Samples *a, b* and *c* were submitted to 4 hours of solvent evaporation, while samples *d*, *e* and *f* were submitted to 24 hours of solvent evaporation.

This result suggests that a factor such as the solvent evaporation time at room temperature could be responsible for the difference in the size of these particles, since all six formulations were prepared with almost the same oil / polymer ratio, 1/5, 1 / 4 and 2/5, respectively, considering the pairs a/d, b/e and c/f. The particle size corroborates the literature[11], which mention that whichever method is adopted to prepare polimeric nanoparticles, generally the size of the particles varies between 100 and 300 nm.

It is worth mention that in some cases, the size of particle may be less than the minimum limit of the described range due to choice of oil, which can modify the characteristics of viscosity and hydrophobicity among other aspects[11]. In this study, neither of the two characteristics was evaluated because just the citronella essential oil was used.

Associated to the particle size measures, the PDI values of each formulation are presented in Figure 4.

The PDI values shown in Figure 4 to all six formulations, are close to 0.3, which represents a moderate and relative homogeneity in the distribution of particle size. Table 2 presents the values of ZP of micro/nanoparticle formulations.

Table 2 shows the values of ZP for the formulation a to f. It could be seen that formulations a to c presents ZP near to the -15 mV, while formulation d to f, around the -10 mV, with some deviation of this value for formulations d and e. According to the literaure, the higher the value of the ZP (less negative) the greater the amount of particle greater aggregation trend[12,13]. In this respect, nanoparticles with ZP above ± 30 mV are stable suspensions which prevents the aggregation of nanoparticles[14].

Characterization of bacterial cellulose membranes containing micro/nanoparticles with essential oil of C. nardus

Figure 5 shows the micrographs of the bacterial cellulose membrane impregnated with micro/nanoparticles of PCL containing citronella essential oil.

The micrographs in Figure 5, from dry and wet cellulose, does not show the bacterial cellulose fibers, except in the sample BCM-33, but with 11,000 times of magnification,

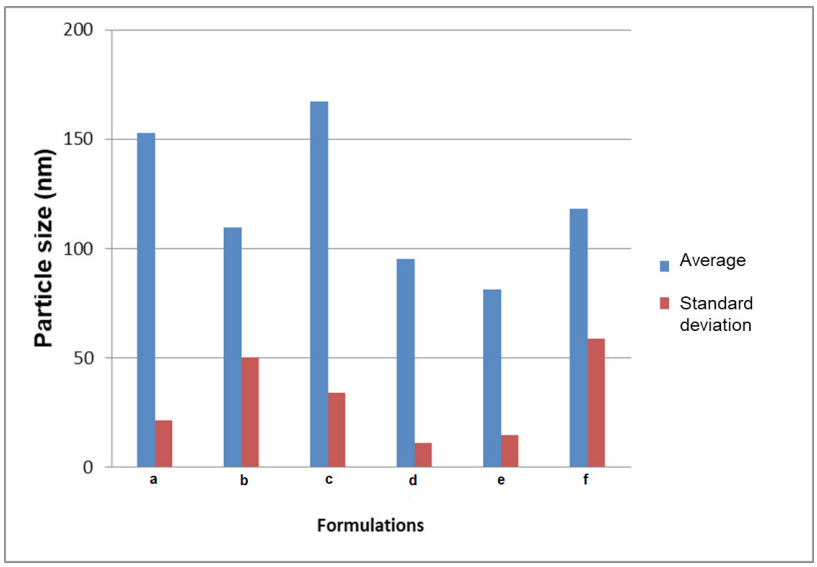

Figure 3 - Particle size of the formulations *a* to *f* measured by DLS.

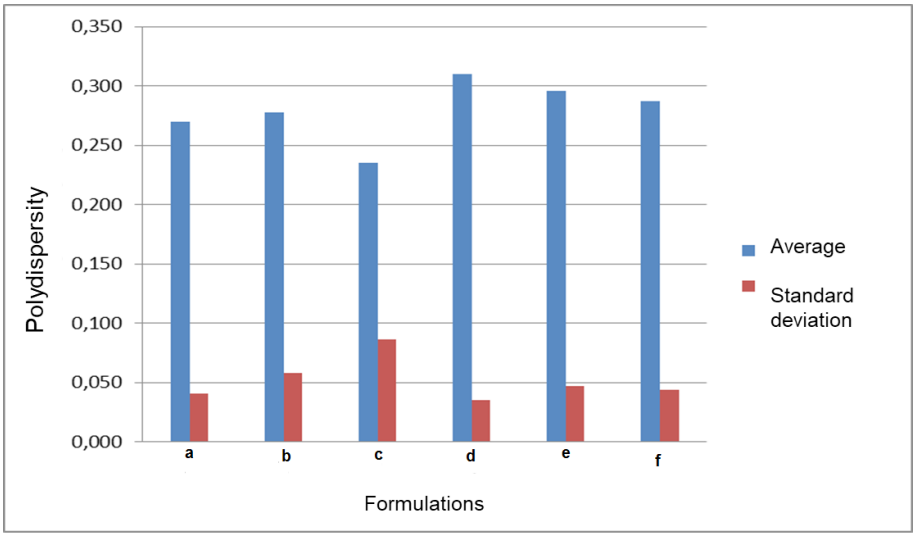

Figure 4 - Polydispersity (PDI) of the formulations a to f measured by DLS.

PZ (mV)		
Formulation	**Average**	**Standard Deviation**
a	-13,940	9,74
B	-14,710	6,76
C	-14,940	5,06
D	-8,71	7,69
E	-7,18	8,98
F	-12,31	8,94

Table 2 - Zeta potential (PZ) of 1, 2, 3 formulations, 4, 5 and 6.

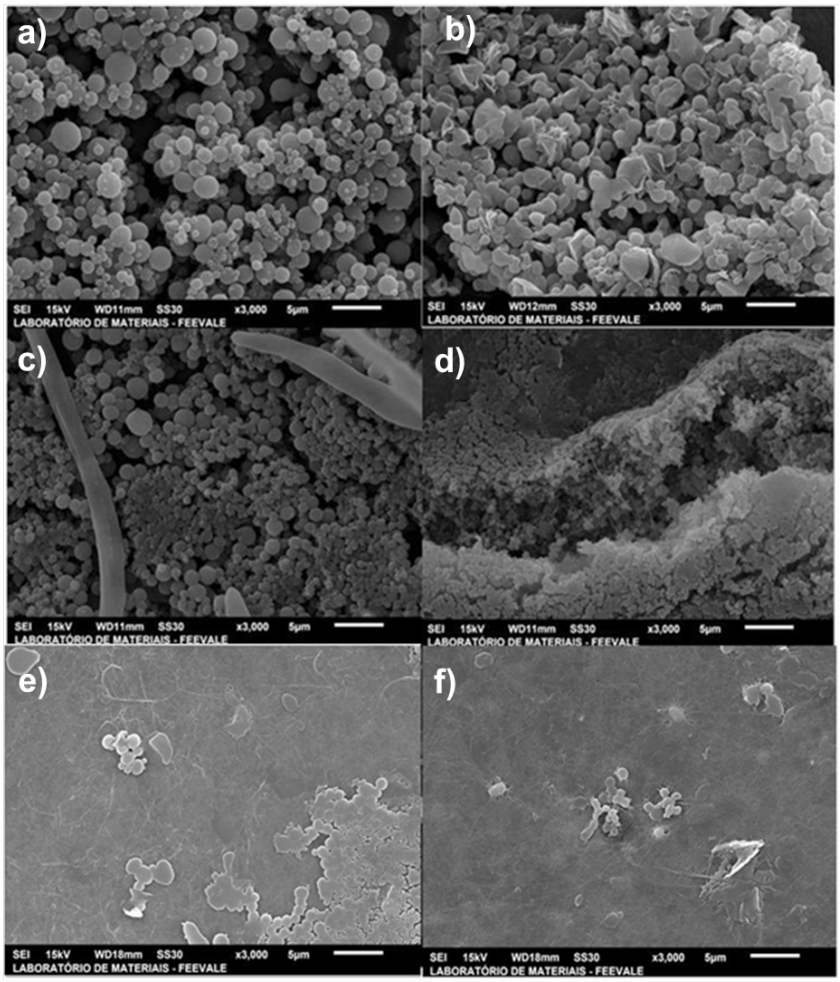

Figure 5 - Micrograph (a) BCD-3 (b) BCM-3 (c) BCD-33, (d) BCM-33, BCD-12 (e) and (f) BCM-12, 3000 x.

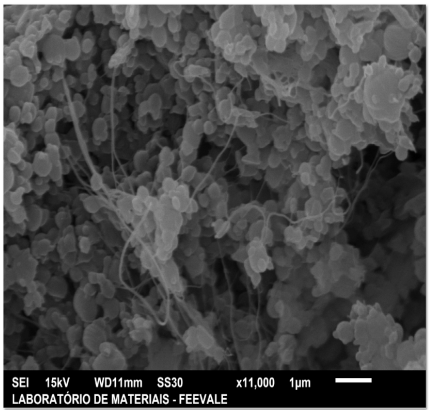

Figure 6 - Micrograph of sample BCM-33 (11,000 x).

as in Figure 6, possible to verify the presence of filaments, suggesting be CB fibers with micro/nanoparticles.

To analyze the presence of fibers between the micro/nanoparticles, Figure 7 shows the cross-section of the BCD-3 samples, BCM-3, BCD-33, BCM-33, BCD-12 and BCM-12 with magnification of 11,000 x.

In Figure 7, is possible to observe the deposition of micro/nanoparticles on the surface of the membranes (a) BCD-3, (b) BCM-3, (c) BCD33, (e) BCD-12 and (f) BCM-12. The cross section images, do not make clear the presence of micro/nanoparticles in the middle of the membrane fibers. Already in the sample (d) BCM-33 is possible to noted the presence of micro/nanoparticles between the fibers of the BC. In this case, the moist membranes allow more easily a deposition and, apparently, less locally thick, giving the impression of absorption of the dispersions.

PCL is a hydrophobic polymer that has application in preparation of hydrophilic polymer composites as BC[14]. The BC has great affinity with polar solvents like water, and lends itself to the preparation of composites, with, for example, PCL. It is suggested, therefore, that the absence of micro/nanoparticles in the BCD-3 samples, BCM-3, BCD33, BCD-12 and BCM-12 is not connected

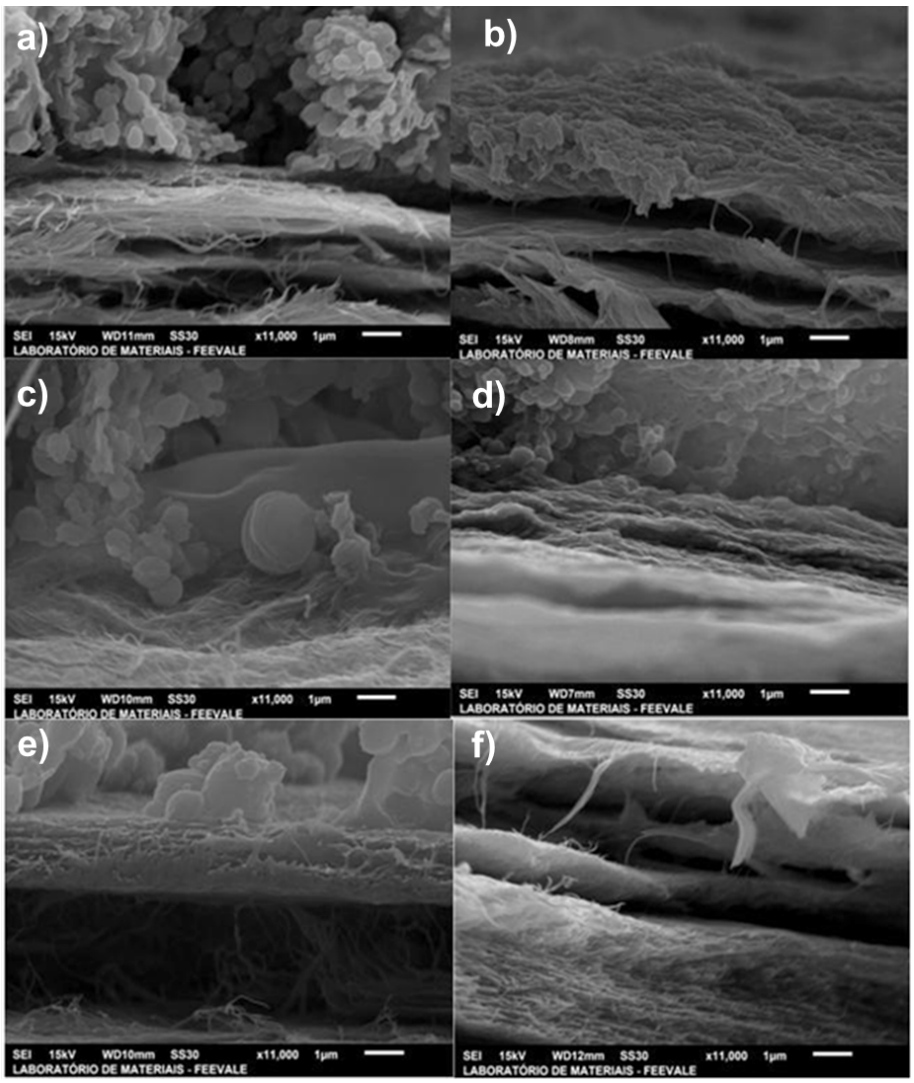

Figure 7 - Micrograph Cross (a) BCD-3 (b) BCM-3 (c) BCD-33, (d) BCM-33, (e) BCD-12 and (f) BCM-12, 11000 X.

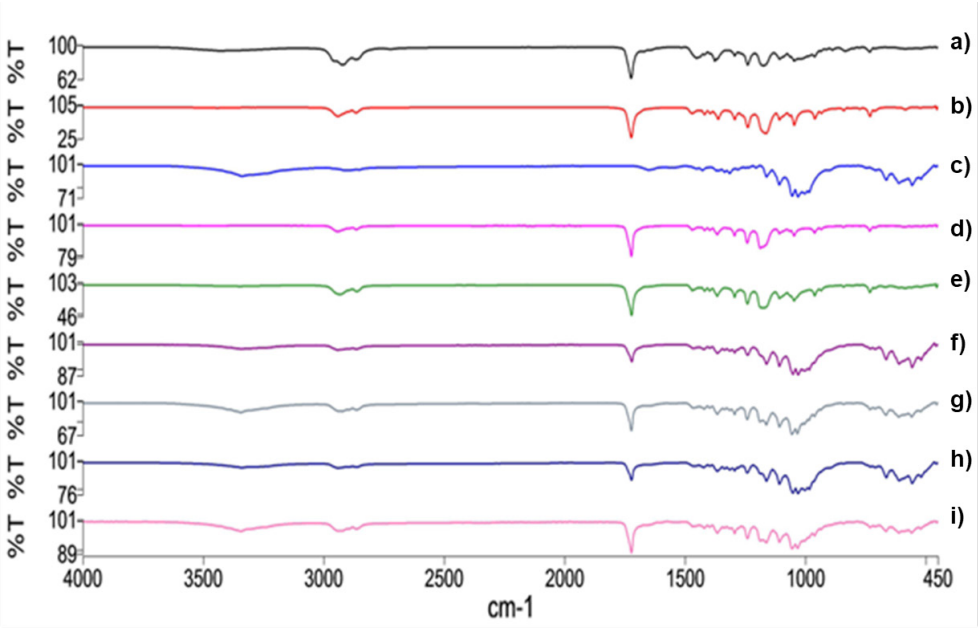

Figure 8 - Infrared spectra: (a) essential oil, (b) PCL, (c) BC, (d) BCD-3 (e) BCM-3 (f) BCD-33 (g) BCM-33, BCD-12 (h) and (i) BCM-12.

of BC. This formulation impregnated on BC in wet form using impregnation aid agent ethanol was the sample that presented the most satisfactory result, micro/nanoparticles between the fibers of the BC.

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New insights into bacterial cellulose materials: production and modification strategies

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ABSTRACT

Komagataeibacter xylinus cultures produced a high amount of bacterial cellulose (BC), which structure consists in a nanoporous network of interlaced fibers. When the culture is performed under static experimental conditions, a membrane with characteristics of highly hydrated hydrogel and good mechanical properties is obtained with promissory applications in the biomedical field. Bacterial cellulose films can be used for many application such as dermal dressing, scaffolds for tissue regeneration and even as a controlled drug release system. Besides, stirred cultures of *K. xylinus* produced amorphous cellulose structures dispersed in the medium with physical and mechanical characteristics different from the membrane. In addition, new properties of BC can be obtained or added if the hydrogel is mixed with other compounds or modified post-purification using both organic and inorganic compounds.

Introduction

Hydrogels are networks shaped by hydrophilic polymer chains which exhibits the ability to swell and retain a significant high fraction of water in their structure, but do not dissolve in it.[1] The advantage of absorbing and retain high amount of water by hydrogels gives softness, elastic consistency and superficial similarity to living tissue. In addition, hydrogels are permeable to small molecules such as oxygen, nutrients and metabolites.[2]

Particularly, Bacterial cellulose (BC) is classified as a hydrogel composed of polymer chains are made up of β-D-glucopyranose monomers linked by beta (1-4) glycosidic linkages.[3] Hydrogen bridges, either inter-chain or intra-chain, which exist due to the high amount of hydroxyl groups of the sugar skeleton hold the chains together and allow them to associate and entangle each other through a self-assembly process, leading to a three-dimensional structure.[4] Particularly, cellulose produced by bacteria has many advantages over cellulose obtained from vegetable sources. The cellulosic material obtained from plants has complex and heterogeneous structures. Plant cellulose is intimately associated with other polymers such as lignin and hemicellulose building complex morphologies.[5] These accessory polymers have specific functions in the physiology of plants. However, for biomedical purposes the plant cellulosic material is required to be intensively purified. In addition, purification processes for vegetable cellulose involve complex and quite expensive mechanisms. For example, mechanical treatments and chemical pre-treatments are often used, which consume a lot of electrical energy and high concentrations of acids and bases, both environmentally pollutants.[5] Meanwhile, bacterial cellulose is produced in a pure form, being the purification process simple, economical and friendly with the environment.[3]

The most common BC-producing microorganisms are members of the *Acetobacteriaceae* family, particularly those belonging to the genus *Komagataeibacter* (formerly called *Gluconacetobacter*). They are Gram-negative, strict aerobic bacteria.[3] The production of BC in liquid media under static culture conditions shows an extensive membrane covering the air/liquid interface of the culture. However, BC is synthesized dispersed in the liquid medium if the liquid culture is stirred during the bacterial growth. The resulting BC structures are generally irregular spheres and/or suspended fibers. The choice of any of these cultivation strategies depends on biopolymer application.[6]

One of the most interesting characteristics of BC is that it can be modified in different ways through environmentally friendly techniques. Unlike other polymeric materials, the processes of production and modification of BC are considered Green Chemistry procedures because of they can be carried out without the use of organic solvents and/or toxic molecules and without any other compound that contaminates the environment. The main strategies of BC modification can be divided into two types: *in-situ* modifications where exogenous materials, such as polymers, are added to the culture medium. The cellulose fibers self-assembled as they are being synthesized, and the exogenous material is incorporated to the network by interacting with the BC fibers. At the end of the process, a hybrid BC

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fiber network with physicochemical characteristics different from those found in a native BC network is obtained. These new characteristics are contributed by the exogenous material and by its interaction within the cellulose fibers and involve intimate modifications in the structure of the BC. On the other hand, *ex situ* modifications consist of all those modifications that are made to the BC after its production and purification process.[7]

The aims of the work are to review the main properties of BC and the "Green strategies" of cellulose modifications in static and agitated cultures to obtain different materials with novel properties (*e.g*. BC membranes and amorphous BC).

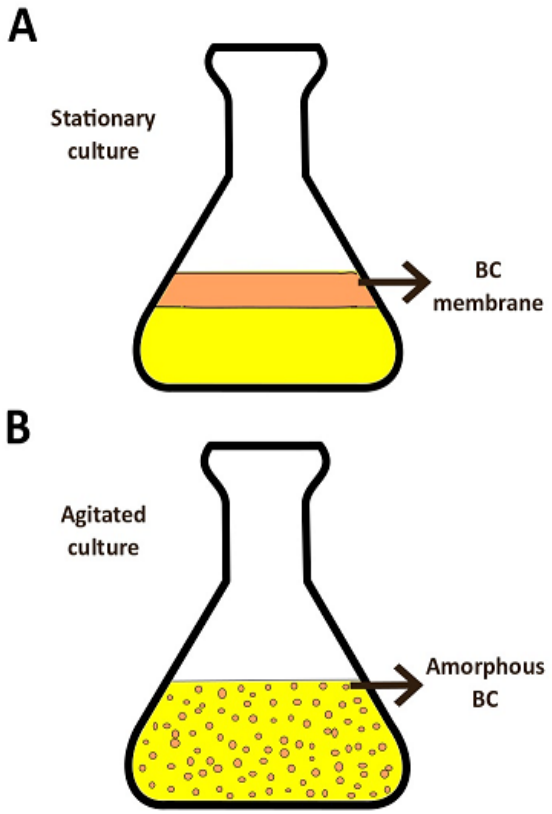

Figure 1 - Cartoon of bacterial cellulose synthesized in (**A**) static and (**B**) agitated cultures.

BC production

The culture of microbial species of *Komagataeibacter* genus and the production of bacterial cellulose (BC) constitute a biotechnological process, which depends on many intrinsic factors such as the bacterial strain, culture medium (mainly carbon and nitrogen sources) and extrinsic factors such as environmental (*e.g*. temperature, pH, etc.).

Specifically, *K. xylinus* species can use diverse carbon sources from monosaccharides (5 and 6 carbons length chain) to oligosaccharides and polymers (*e.g*. starch), or other molecules such as alcohols and organic acids.[8,9] However, the BC yield will depend on the metabolic pathway of each carbon source. For example, supplementation with ethanol to media containing glucose results in an increase in cellulose productivity, which could be partially attributed to the increase of cellular membrane permeability.[10,11] In a recent work, the authors reported an increase in more than 500 BC yield by supplementing with 1% of rapeseed oil to Herstin-Schramm medium (containing 2% glucose and 0.115% citric acid as main carbon sources). The authors also claimed an increase of 285% BC thickener and correlated with high tensile strength compared to the control without oil supplementation.[12]

Regarding the nitrogen sources, the highest yields are given for the combination of yeast extract and peptone in the culture medium.[11] On the other hand, the optimum pH and temperature for cellulose production using *K. xylinus* was found in the range of 5.0-7.0 and 28-30°C, respectively.[13,14]

K. rhaeticus isolated from kombucha tea was able to produce BC in a Hestrin and Schramm medium partially o totally supplemented with sugar cane molasses, reducing the BC production costs up to 20%, a critical point for BC production at large scale.[15] Following the same line of work, *Gluconacetobacter sucrofermentans* B-11267 was able to synthesize BC in media containing acid food by-products such as cheese whey at pH= 4.96 and thin stillage at pH= 3.95, 5.0 and 6.0. The BC production showed an increase of 2.5 to 3-times compared with bacterial cultures in Hestrin and Schramm medium under similar experimental conditions. X-ray analyses of BC films showed a change in the BC microfibril width and crystallinity but without changing its chemical structure.[16]

The biochemical process of BC synthesis consists of three main steps: (i) polymerization of glucose residues in the β-1-4 glucan chains, (ii) extracellular secretion of linear chains through pores or terminal complexes of the bacterial cell (CTs) with 3.5 nm in diameter and (iii) organization and crystallization of glucan chains in hierarchical arrangements due to hydrogen bridge interactions and van der Waals forces.[6] The nascent chains form 1.5 nm subfibers wide which in turn are assembled into nanofibers of 2-4 nm in diameter. Then, these nanostructures are associated in more complex structures in the form of films of 40-60 nm wide and with a thickness of 3-8 nm that can intermingle and entangle forming an exceptional 3D network that gives rise to the membranes or films (Figure 2).[3] BC in these membranes, the crystalline strucutre is higher than 70%.[17]

Static cultures

Static cultivation of *K. xylinus* in liquid media (**Figure 1A**) produces floating cellulose membrane at the interface liquid-air that helps the bacteria to have large availability of oxygen.[18] In addition, the BC membrane protect the bacteria from other microorganisms in the environment and works as a physical barrier against UV radiation and redox processes. Also, BC membrane increases the ability to colonize other places and maintains a hygroscopic environment avoiding periods of dehydration and lack of moisture.[19, 20]

Agitated cultures

When *K. xylinus* is cultivated under constant agitation it is not possible to obtain a membrane as in the static culture, but cellulose synthesis is observed as small spheres and/or amorphous agglomerations (**Figure 1B**). The BC yields in these types of cultures are lower than those of the static culture because of the growth of non-BC producing bacterial cells which are competing and consuming the substrates. The decrease of BC yields was attributed to the accumulation of mutations that damage the machinery responsible for polymerizing glucose.[21-23]

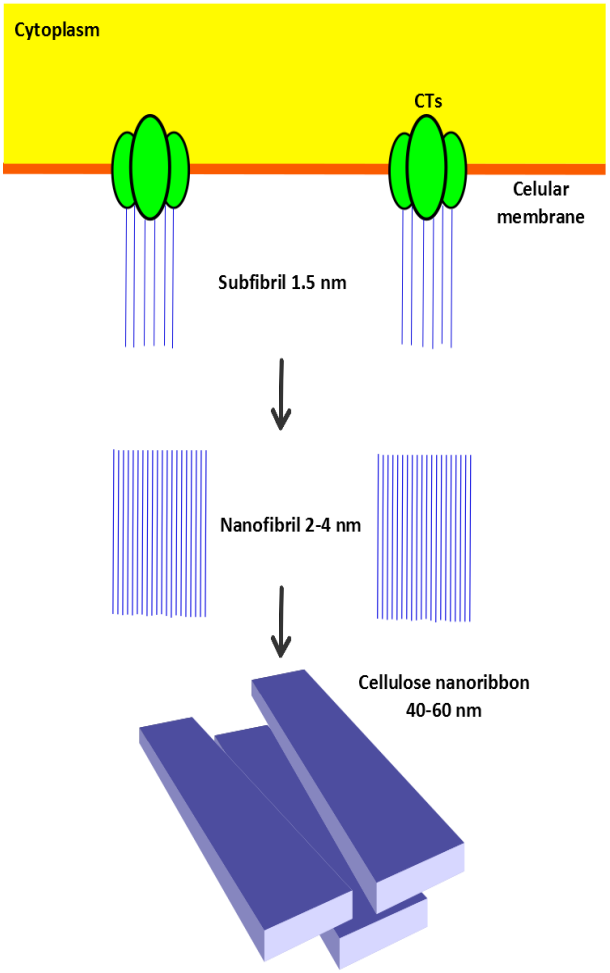

Figure 2 - Bacterial cellulose biosynthetic and self assembly representation of network (modified from Cacicedo *et al*., 2016).

Additionally, a large amount of glucose is converted to other molecules such as gluconic and keto-gluconic acids that are released into the medium. The deviation of carbon source utilization by alternative metabolic pathways leads to the synthesis of other metabolites causes a detriment in the cellulose production.[22,23] A comparative study reported at least 3-times lower BC yield, from 28 g/L and 9 g/L of BC obtained in static and agitated cultures respectively.[23] Also, strong changes of BC morphological and crystalline structure under static and agitated cultivation were reported.[22,23] In both studies, the reticulated structure of fibers is maintained in both culture methodologies but with subtle changes, since the fibers presented a superior curvature in the agitated cultures and they were more entangled among themselves giving rise to a denser reticulated matrix. These changes are supposed to be related to variations at the BC microstructure level, such as the degree of polymerization, the crystallinity and the relationship between the *Iα* and *Iβ* cellulose allomorphisms.[22,23] According to their reports both the crystallinity and the size of the crystals are diminished under conditions of agitated cultivation. The reports also found that the BC *Iα* allomorphism decreases and the more stable BC *Iβ* increases, concomitantly with the degree of polymerization of the glucose chains, which is reduced in compared with the static culture.

BC properties

Bacterial cellulose matrices possess unique properties, such as:

• Ability to retain a large amount of water, up to 99% of its content, which exceeds cellulose obtained from plant sources. A physical type hydrogel is thus formed.[5]

• High crystallinity and high degree of polymerization that confer excellent mechanical properties, superior to vegetable cellulose. The tensile strength of the BC is usually between 200 and 300 MPa, and its Young's modulus is between 15 and 35 GPa.[24]

• High thermal resistance due to its high degree of purity and crystallinity. This property is very important for biomedical applications because allows the biomaterial to be thermally sterilized.[25]

• Biodegradability that classifies BC as a green material.[26] BC is not be able to be degraded by mammalian cells because of lacking 1,4-ß-D-gluco-hydrolase activities. Meanwhile, BC can be hydrolyzed by several microorganisms in the human gut and in the environment with the ability of express enzymes capable of break 1,4-ß-D-glycosidic linkages.[27]

• Excellent biocompatibility. *In vivo* studies of subcutaneous implantation of membranes in rats showed that they do not produce fibrosis or granulomas after 12 weeks, which shows that there is no reaction to foreign bodies. In addition, there was no redness, swelling or edema around the site of implantation site.[28]

• The BC membranes are asymmetric. The surface of BC in contact with the air are very closed polymeric network with narrow size porosity. Meanwhile, the BC surface in contact with the liquid media displays pending cellulose chains because of the bacterial cell synthesis and able to be easily tailored applying different strategies.[3]

Another relevant aspect related to the production of membranes is the versatility of BC production. The shape and thickness can be easily controlled by varying the mold type of the reactors and the cultivation time.[3,29]

The unique properties of the BC have inspired its use in numerous commercial products, including strips, headphone membranes, high quality paper, dietetic foods and textiles. However, the most promising properties of BC are

found in the biomedical field for multiple purposes such wound dressing, synthetic skin, scaffolds for tissue engineering, artificial blood vessels, controlled drug release systems and dental implants.[3,5,29]

Modification of BC

Although, the choice of BC production methodology possesses the advantage of tailoring its properties creating new ones that native BC does not have. The simplest procedure to tailor BC is to incorporate a non-degradable molecules/structures in the culture creating a hybrid BC structures. Exogenous material can be polymers, nanoparticles, metals, metal oxides, clays and solid particles of macrometric size. There are two main ways of convert native BC into hybrid materials using Green Chemistry techniques which will be described below.

In-situ modification

In this procedure the reinforcing material is added to the culture medium at the beginning or during the biosynthetic process.

This technique is often accompanied by significant changes in the BC structure, higher than other strategies, giving rise to compounds with physicochemical characteristics and distinctive properties (**Figure 3**).[7]

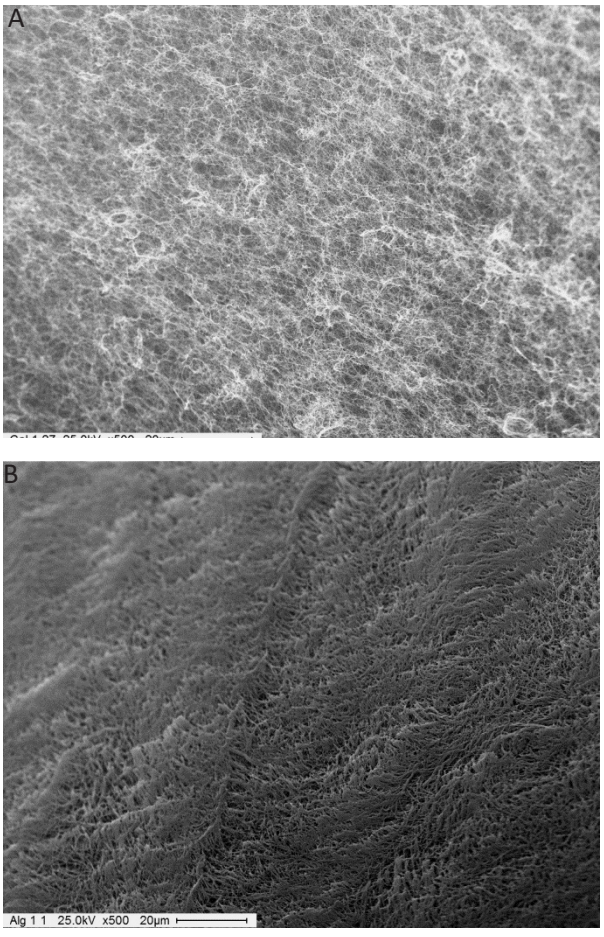

Figure 3 - Scanning electron microscopies of native bacterial cellulose (A) and Alginate-Bacterial cellulose composite (B) [7]

This technique is often accompanied by significant changes in the BC structure, higher than other strategies, giving rise to compounds with physicochemical characteristics and distinctive properties (**Figure 3**).[7]

Several BC compounds have been generated from this type of methodology by the addition of different compounds such as polyvinyl alcohol, graphene oxide, carboxymethylcellulose, alginate and even an extract obtained from *aloe vera*, obtaining characteristics and properties very different from those of the materials separately.[7,30-33]

Ex-situ modification

In this method, the BC can grow conventionally, and after purified the membranes are mixed with the reinforcing material. This technique is very versatile and simple, and the most important factor when choosing it is that the original structure of the BC remains almost unchanged because the exogenous material added does not interfere with the assembly of microfibrils in *de novo* synthesis. The integration of the material depends on its size and chemical nature, so only submicrometric and nanometric materials can interpenetrate the network in a homogeneous way because they fit in all its pores; and non-polar materials would not be combined with the membrane.[7]

This strategy has successfully incorporated numerous compounds on BC matrices, including chitosan, silica, silk, silver nanoparticles, phosphate microparticles and even clay minerals.[34-41] The hybrid materials formed showed an alteration in the properties in relation to the native BC (**Figure 4**).

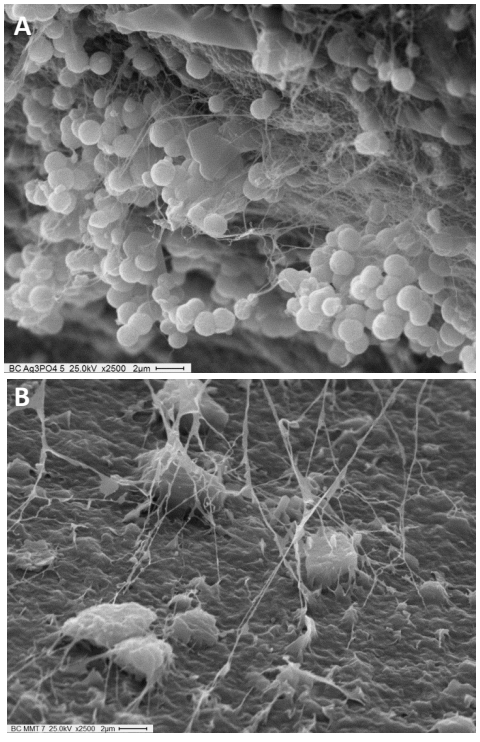

Figure 4 - Scanning electron microscopies of Ex-situ bacterial cellulose modified with silver phosphate (A) and Montmorillonite (B) microparticles respectively.40, 41

Conclusions

The cultivation of bacterial species of the *Komagataeibacter* genus and the manipulation of different parameters of the biotechnological production process allows the obtention BC with different morphologies, dimensions, and topologies according to the application that is wanted to be given to the biopolymer. If the culture is performed statically, a membrane is produced at the interface between the liquid and the culture medium. The highly hydrated character, the good mechanical properties and the biocompatibility make these membranes a promising material in biomedicine, where it has diverse applications. However, if the culture is stirred during the incubation period the cellulose produced has a morphology of spheres and/or fibers dispersed in the culture medium. The versatility with which the BC is produced is one of the main causes of its wide range of applications. Most of the research works carried out have sought to study the applications of cellulose produced in the form of a membrane by means of static cultures. Besides, there is a vacancy in the study of agitated cellulose cultures and their biophysical properties under these experimental conditions.

On the other hand, the two modification strategies of the BC membranes allow to obtain hybrid materials by soft techniques with novel and optimized properties for the different applications. *Ex-situ* modification has proven to be a versatile, simple and very useful modification method when the exogenous component is toxic or unstable to or in the bacterial culture or in the presence of the growing membrane. In contrast, *in-situ* modifications are more complex processes and the added material must be compatible and able to remain in solution or suspension in the culture medium during BC membrane growth. However, the degree of modification on the cellulose membranes by *in-situ* is higher and the presence of the external component in the hybrid material increased.

Finally, bacterial cellulose continues to be present as a valuable biomaterial for several applications. Even though in recent years has increased the research in the BC field, it is relevant to mention that there are still some vacant areas of BC production under diverse experimental conditions to be explored. Consequently, it is essential to explore how these changes could affect the structure, composition and biophysical properties of diverse composite BC materials.

Acknowledgments

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▷Biomaterials - Abstracts

1º Encontro Brasileiro de Biocelulose

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Monitoring of bacterial cellulose degradation under different conditions: SOIL, SEA, natural weathering and accelerated aging

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ARTICLE INFO

ABSTRACT

Bacterial Cellulose (BC) is a highly crystalline linear glucose polymer synthesized extracellularly in the form of nanofibers by a large number of organisms, but the most commonly used bacterium is *Komagataeibacter hanseni*. Although the BC has great potential for many applications, the development of new materials implies knowing the environmental impact that it can cause. Thus, this work aimed to evaluate the degradation of BC in different environmental conditions. BC membranes were synthesized by *K. hansenii*, later purified in sodium hydroxide solution and dried in an air circulation oven. The degradation of the membranes was evaluated at different times in the following environments: soil (SO), estuarine environment (SEA), natural weathering (NW), and accelerated aging chamber (AAC). The samples were characterized by visual analysis (VA), Fourier transform infrared spectroscopy (FTIR/ATR) and thermogravimetric analysis (TGA). In the visual analysis it was possible to evaluate the physical alterations, such as: roughness, cracks, and color change. The results showed that the membrane degradation kinetics occurred in the following order: SO>SEA>NW>AAC. It is believed that in SO and SEA the degradation was more intense due to the presence of microorganisms and humidity in these environments. The total degradation of these membranes occurred in 5 days and 15 days respectively. The samples submitted to NW despite being exposed to factors such as wind, rain and radiation extended their degradation to 90 days. The CB membranes exposed in AAC had a slower rate of degradation compared to other environments influencing the mass and morphological properties.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Nisin In Bacterial Nanocellulose: An Antimicrobial Activity Evaluation

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ARTICLE INFO

ABSTRACT

Nisin is an antimicrobial peptide, 3.4 kDa, produced by the microorganism Lactococcus lactis (ATCC 11454). This bacteriocin inhibit the development of Gram-positive bacteria and Gram-negative bacteria, in a presence of chelating agents. Bacterial nanocellulose (NcB) has been considered an ideal and high-quality material applied in food, medical and pharmaceutical supplies. Due to all these benefits presented it is important to know the behavior of the NcB system containing nisin. For this reason, NcB were placed in a 24-well plate and 1 mL of nisin solution (0.1g.mL-1 with activity in 5 log10 AU.mL-1) was added in each well. The plate was kept on a rotating shaker at 30 °C, 100 rpm for 4 h. The nisin amount loaded in NcB was analyzed through protein assay. The antimicrobial activity against the microorganisms Staphylococcus aureus, foodborne pathogen, and Lactobacillus sakei (nisin bioindicator) were analyzed during 180 days by agar diffusion assay in different temperatures (4 oC, 25 oC and 37 oC). The results indicated the nisin was loaded in NcB, around 700ug.mL-1 with 6 log10 AU.mL-1, antimicrobial activity increased 1 log10 AU / mL. The antimicrobial activity results showed the system NcB-nisin was capable to inhibit the both microorganisms' growth, up to 60 days. The system showed good efficacy and the NcB potentiated the antimicrobial action of nisin, acting as a selective barrier of other compounds present in the standard solution and, as protection to the different temperatures. Indicating that NcB may be an ideal system for nisin and other compounds.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Clinical evalutation of the bacterial cellulose membrane to the surgical treatment of deep corneal ulcers in dogs and cats

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ARTICLE INFO

ABSTRACT

Introduction: Deep corneal ulcers in companion animals constitute ophthalmic emergencies and, almost always, require surgical treatment that, if not adopted, leads to eye loss and blindness. In this sense, the use of the bacterial cellulose membrane embedded by ciprofloxacin (BCMF) in animal's cornea healing with corneal ulcers was clinically evaluated in this work. Methodology: Five dogs and one cat that had deep corneal ulcers and feline ulcerative keratitis by corneal sequestrum were evaluated, respectively. All received BCMF under the usual technique of keratoplasty. All animals were prepared for prior anesthesia care and routine clinical treatment of ulcers. The BCMF were cut to the same dimensions of the lesion beds and applied using a suture pattern interrupted with 9-0 nylon suture wire. Routine postoperative clinical measures were adopted for up to 21 days. Results and discussion: For all animals, immediate improvement of the ocular pain sign was observed. There was corneal vascular exaltation at 15 th postoperative days and its gradual attenuation after 30 th postoperatively. In two patients (dogs), dryness of the membrane surface was observed and need to the trimmed in an additional surgical maneuver at 40 th postoperative day. It was observed the occurrence of cicatricial leukoma next to the grafting area, but no signs of extrusion were observed. The transparency of the córnea around leukoma was restored. Conclusion: The results allow to admit that MCBF may be a therapeutic alternative in the surgical treatment of ulcerative keratitis in animals, however, additional studies with a greater number of patients are necessary to corroborate this clinical observation.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Use of bacterial cellulose-based hydrogel for wound healing after diode laser exeresis of sarcoid face in equine – case report

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ARTICLE INFO

ABSTRACT

Introduction: Sarcoid is a cutaneous neoplasm in equines. The use of surgical diodo laser has indication in neoplasias for promoting immediate hemostasi. BC-based hydrogel consists on a formulation for wound repair that increases fibroblast proliferation. We report the case of an equine with sarcoids treated with diode laser exeresis associated topic use of BC-basec hydrogel. In addition, in postoperative period the wound healing process was evaluated using BC-based hydrogel. Methodology: An equine, Quarter Horse, male, castrated, 9-years-old, 460 kg, attended at the Veterinary Teaching Hospital of UNESP/FMVA, presenting multiple sarcoids, with a fibroblast sarcoid on the left side of face (9 cm 2), with exacerbated growth for 2 years. Treatment consisted in diode laser exeresis of 4400mW, 4J and continuous frequency. The postoperative period consisted of application of phenylbutazone (4.4 mg/Kg/IV/q24h) for 3 days, and enrofloxacin (5mg/Kg/IM/q24h) for 10 days. Daily dressings (q12h) were performed using topical iodopolividone and repellent application. However, after 24 days without favorable evolution of cicatrization, with necrosis, BC-based hydrogel was applied daily on the wound after cleaning with saline solution. Results and discussion: From the use of the BC-based hydrogel, healing progressed favorably, so that after 6 days the wound was completely covered by granulation tissue, with evident contraction and epithelization. After 40 days the wound was fully epithelialized. Conclusion: It was concluded that diode laser exeresis was effective in the treatment of this sarcoid and the association with BC- based hydrogel was effective in the granulation, contraction and epithelization phases characterizing the wound healing.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Nexfill ® dressing for lower limb ulcer healing in diabetic patients

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ARTICLE INFO

ABSTRACT

Chronic wounds represent a public health problem due to the number of people affected and due to their chronic nature, they are responsible for the main cause of lower limb ulcers, resulting in a compromise in the quality of life of patients affected by these wounds. One of the ways of treating chronic wounds is to use bandages composed of bacterial cellulose. These dressings have biocompatibility in vivo and when applied in cutaneous wounds provide healing more effectively. This paper aimed to report a case study of a patient living in Shelter of Elderly Dona Helena Dornfeld, in São Carlos / SP, who received the treatment promoted by a multidisciplinary team composed by nurses, nutritionist and physiotherapist using the Nexfill® dressing. A 93-year-old female patient with a diagnosis of Alzheimer' s and Type II Diabetes, with a lower limb wound there are more than 6 months. The treatment consisted of cleaning the wound with physiological solution and then the initial application of Nexfill ® dressing, replacements every 5 days. Images were recorded every 30 days and the área medication was determined using ImageJ ® software. After the 60-day period, it was possible to observe that the Nexfill ® dressing made possible the complete healing of the wound. Acknowledgments: To the team of Shelter of Elderly Dona Helena Dornfeld.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Bacterial cellulose aerogels: influence of functionalization on mechanical and absorption properties

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ARTICLE INFO

ABSTRACT

Cellulose represents one of the most attractive classes of materials for innovative applications and has been used as a raw material in the elaboration of products, including nanostructured ones, such as nanocomposites and aerogels – which are solid materials that have high porosity, low density and large area surface – where its properties have encouraged the emergence of new high value applications. To expand the range of applications, some studies have investigated the functionalization of aerogels. In the present work, in order to study the impact of functionalization on aerogels properties, bacterial cellulose (BC) was carboxylated by TEMPO-mediated oxidation, nanofibrillated in blender, silanized with methyltrimethoxysilane, and the final suspension was frozen in N2 liquid and lyophilized. Oxidation and silanization were evidenced by FTIR. Aerogels were produced from this functionalised suspension (BCOXNS) and compared to other non-oxidized aerogels, namely BCN (nanofibrillated BC), BCNS (nanofibrillated and silanized BC) and BCOXN (oxidized and nanofibrillated BC). Silanized aerogel presented a morphology with an organized lamella structure formed by the microfibrilar network. BCOXNS aerogel presented lower liquid absorption capacity than BCN and BCNS, but higher mechanical properties, which allowed its use during seven absorption-drying cycles. BCOXNS aerogel presented the best set of properties and was used as a model for future studies with other raw materials.

1º Encontro Brasileiro de Biocelulose

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Biosynthesis and characterization of bacterial cellulose by komagataeibacter from different culture conditions

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ARTICLE INFO

ABSTRACT

Bacterial Cellulose (BC) production is traditionally conducted from commercial culture media containing glucose as the source of carbon and other high-cost nutrients for the process. The use of carbon and nitrogen sources from agro-industrial waste is an alternative to lower costs in obtaining biotechnological products beyond to lessening the impacts on the environment caused by the inappropriate disposal of this material. In the present work, we evaluated the production and characterization of bacterial cellulose (BC) films obtained by *Komagataeibacter hansenii* ATCC 23769 culture using mannitol, glucose, fructose, lactose, glycerol, inulin and sucrose as alternative carbon sources, corn steep liquor (CSL) and Prodex Lac® as nitrogen sources. The gelatinous membrane formation of CB was monitored for 12 days, under static condition and temperature of 30 ºC. The membranes were purified by different methods, showing the same influence on the thermal and chemical properties of the obtained material. After purification the membranes were dried and characterized by scanning electron microscopy (SEM), thermogravimetric analysis (TG) and Fourier transform infrared spectroscopy with attenuated reflectance accessory (FTIR/ATR). The highest concentration of BC was found in culture medium containing Prodex Lac® as the nitrogen source. Among the sugars, lactose, fructose and mannitol presented the best results. TG analyzis indicate that all membranes have similar thermal behavior. FTIR results show that chemically all membrane samples (despite nitrogen or carbon source) are equivalent in structures. The micrographs have shown that the medium may influence the morphology of CB, but in general, all presented nanofibers, an important feature in the membrane.

1º Encontro Brasileiro de Biocelulose

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Bacterial cellulose membranes for food active packaging

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ARTICLE INFO

ABSTRACT

The main function of food packaging is to preserve the maximum quality of the product, aiming to increase shelf life. Conventional packaging is slowly losing space for "active" and "smart" packaging that interact directly with the product. Thus, the objective of this work was to produce and characterize BC membranes incorporated with rosemary (20% and 100% -pure) and gorgonzola aromas, and silver nanoparticles (NpAg) for possible application as an active package to extend shelf life of various food products, improve their quality and intensify their sensorial characteristics. BC membranes were synthesized by Komagataeibacter hansenii bacteria in a static culture at 30ºC for 12 days, after purified, incorporated with the scents and NpAg through soaking and later dryed. After, the membranes were characterized by Fourier transform infrared spectroscopy with attenuated reflectance accessory (FTIR/ATR) and thermogravimetric analysis (TGA). The FTIR/ATR analysis indicated the NpAg, and the gorgonzola and rosemary20% scent was incorporated in the membrane, differently from what happened with the pure rosemary scent. In this case, the rosemary scent was probably not incorporated to the membranes, because of its oil nature. The thermal stability was reduced to 38.2 °C, 24.2 °C, 1 °C, and 13.4 °C for BC/NpAg, BC/gorgonzola, BC/rosemary100%, and BC/rosemary20%, respectively, according to the TGA analysis. The antimicrobial property was proven for the membranes incorporated with the rosemary (20% and 100%) scents and NpAg. As for the membrane incorporated with the gorgonzola scent, there was no growth also, but this result was attributed to other substances used as vehicles thereof.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Evaluation of soy molasses as fermentation medium for bacterial cellulose production

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ARTICLE INFO

ABSTRACT

Bacterial cellulose (BC) is a biopolymer with application in several areas; however, its large-scaleproduction is limited due to the high cost of the process and the fermentation medium. The soy molasses (SM) byproduct canbe a promising substrate for BC production, since it can serve as carbon and nitrogensource required for microbial development. In order to determine the potential of soy molasses to produce bacterial cellulose, an aqueous solution containing 75 g/Lof SM hydrolyzed with 5% (v/v) 1M H2SO4was heated at 90 °C/10min (mediumSMH75). Aliquots of 50 mL of MSH75 and HS medium (reference medium) were distributed in culture flask and sterilized at 121 °C/15min. After sterilization, ethanol (0.0, 1.0, 1.5 and 2.0% v /v) was add to the mediaand inoculated with 10% (v/v) of *Komagataeibacterxylinus* ATCC 53582culture. The fermentation was conducted at 30 °C/10 days, under static condition. The obtained membranes were characterized by Fourier Transform Infrared spectroscopy (FTIR), thermal gravimetric analysis (TGA), and X-ray diffraction (XRD). The supplementation of MSH75 medium with 2.0% ethanol increased BC production in55%when comparedto the unsupplemented medium and lead to a similar production (7.0 g/L) totheHS medium. The membranes obtained in the MSH75 medium supplemented with 2.0% ethanol had typical bands of cellulose, thermal stability and crystallinity similar to those obtained in HS. Hydrolyzed soy molasses supplemented with ethanol presents great potential as a fermentation medium for BC production via static fermentation.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Functionalization of SCAFFOLDS of PLA printed in 3d structure for application in tissue engineering

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ARTICLE INFO

ABSTRACT

Multifunctional nanocomposites Regenerative medicine is an area of medicine aimed to upgrade organs and tissues regeneration process, though the creation of temporary substitutes that is able to guide and stimulate this process. In order to upgrade this process it is necessary the development of biocompatible materials that should work as supports (Scaffolds) interacting specifically with tissues that will be regenerate. The poly acid lactic (PLA) biopolymer is biocompatible and biodegradable, being the material that has come to stand out as an effective scaffold bone repair. The objective of this work is to evaluate the cellularization of the PLA printed in 3D structure, by the technique FDM in the laboratory of Biopolmat of the UNIARA. For this analysis, PLA will have its surface modified by CAP (Col atmospheric plasma) and DLW (Laser direct Writing), and will also be adsorbed to rhBMP-2 (recombinant human morphogenetic protein 2). Presterilized PLA scaffolds are used in a culture of MC3T3 cells (mouse myoblasts) and C2C12 (mouse myoblasts) in 48 well plates for 24 and 48 hours. After the cell adhesion, proliferation and cell adhesion period, it is evaluated by the MTT colorimetric method. The physical and rhBMP-2 induced differentiation at different concentrations and assessed by the perception of alkaline phosphatase activity. The expected results are the surface treatments of PLA with the action of rhBMP-2, improved adhesion, a proliferation and a difference for the use of PLA, which is effectively used in bone regenerative medicine.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Variation of carbon sources for bacterial cellulose production to use as sustained drug release system

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ARTICLE INFO

ABSTRACT

Bacterial cellulose membranes (BC) has received special attention in recent years due to its notable characteristics such as high purity, mechanical strength, crystallinity, biodegradability and biocompatibility, which allows their use in medicine and pharmaceutic areas. Nevertheless, the low yield and the high production cost constitutes a limitation BC use in these areas. The objective of this work was to obtain a higher yield of BC by *Gluconacetobacter hansenii* (ATCC 23729), by varying the type and carbon source concentration (glucose, sucrose and fructose) of the culture media, to use as antibacterial drug release topic system. The BC obtained were characterized by Fourier transformed infra-red spectroscopy, scanning electron microscopy and X-ray diffraction. Disks of the different BC were impregnated with antibacterial drugs, rifampicin (BC-RFM), ceftriaxone (BC-CRO) and levofloxacin (BC-LVX) and tested by Franz cell permeation and disc diffusion. The results presented that BC-RFM showed greater retention capacity and lower velocity of RFM releasewhen compared to BC-LVX and BC-CRO. This result can be related with the fact that RFM molecule is rich in OH radicals promoting the interaction by hydrogen bonds with the OH radicals of CB.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Case study: curative biocellulose in abrasion

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ARTICLE INFO

ABSTRACT

Multifunctional nanocomposites It is a case study of an abrasion wound, motivated by a motorcycle accident, treated with a biocellulose curative with the intention of knowing the acceptance of the product by the user, the benefits and the differentials of this. In the healing process, the with reconstructive phase begins on the fourth day after the tissue discontinuity, coinciding the application of the biocellulose curative Nexfil® in this study were used the smooth and porous versions to treat lesions found in grade I, II and III in the following body extensions: right palmar region - 2 lesions, 5 x 4 cm and another 3.5 x 7 cm; left palmar region - 8 x 8 cm lesion; flank D: lesion 10 x 9 cm; and all digital pulps. The biocellulose film was installed without adequate technical equipment, at home where the patient living conditions did not allow an aseptic environment and that would allow the development of an infectious process. Immediately, the use of the biocellulose film, facilitated the visualization of all the stages of the cicatricial process; the porous version allows the exudate elimination also stands out the accentuated adhesion, supporting baths without detachment and the possibility of dispensing an additional coverage. There was a report by the patient of immediate analgesia after an initial sensation of local warmth perceived during the application of the biocellulose film and which made viable the resumption of patient movement it does not show adhesion in clothes and detaches in a synchronized manner to healing timing.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Functionalization of SCAFFOLDS of PLA printed in 3d structure for application in tissue engineering

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ARTICLE INFO

ABSTRACT

Multifunctional nanocomposites Regenerative medicine is an area of medicine aimed to upgrade organs and tissues regeneration process, though the creation of temporary substitutes that is able to guide and stimulate this process. In order to upgrade this process it is necessary the development of biocompatible materials that should work as supports (Scaffolds) interacting specifically with tissues that will be regenerate. The poly acid lactic (PLA) biopolymer is biocompatible and biodegradable, being the material that has come to stand out as an effective scaffold bone repair. The objective of this work is to evaluate the cellularization of the PLA printed in 3D structure, by the technique FDM in the laboratory of Biopolmat of the UNIARA. For this analysis, PLA will have its surface modified by CAP (Col atmospheric plasma) and DLW (Laser direct Writing), and will also be adsorbed to rhBMP-2 (recombinant human morphogenetic protein 2). Presterilized PLA scaffolds are used in a culture of MC3T3 cells (mouse myoblasts) and C2C12 (mouse myoblasts) in 48 well plates for 24 and 48 hours. After the cell adhesion, proliferation and cell adhesion period, it is evaluated by the MTT colorimetric method. The physical and rhBMP-2 induced differentiation at different concentrations and assessed by the perception of alkaline phosphatase activity. The expected results are the surface treatments of PLA with the action of rhBMP-2, improved adhesion, a proliferation and a difference for the use of PLA, which is effectively used in bone regenerative medicine.

1º Encontro Brasileiro de Biocelulose

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Procedure for obtaining individual kinetic parameters for bacterial cellulose

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ARTICLE INFO

ABSTRACT

One of the purposes of the study of kinetic processes in condensed phase is the determination of thermally stimulated reactions parameters. In general, the analysis methods involve the estimation of kinetic parameters based on the general equation given by:

$$\frac{d\alpha}{dT} = \frac{A}{\beta} e^{-\frac{E}{RT}} f(\alpha)$$

The equation above represents a simple non-isothermal process, involving a reaction governed by a single kinetic mechanism. Meantime, in the case of complex processes may be involved two or more overlapping reactions, being convenient to carry out the separation of the kinetic curves in individual cases. The separation (or deconvolution) of the kinetic "peaks" can be carried out by adjusting of symmetric functions such as the Gaussian function, Lorentzian, Weibull, or Suzuki-Fraser. Fraser-Suzuki (FS) function is a modification of the Gaussian function and has shown better results in describing the asymmetry characteristic of non-isothermal kinetic curves. This study aims to present a separation procedure of the "peaks" of the kinetic curves of processes involving cellulose thermal decomposition and determine the overall activation energy based on individual kinetic parameters E, A and f (α). Therefore, was possible to determine the kinetic parameters of individual reactions and describe the activation energy profile as a function of the degree of conversion of the global process. In logarithmic form, the terms of each individual kinetic equation is given by:

$$ln\left[\frac{FS_i}{f_i(\alpha)}\right] = ln\left[\frac{A_i}{\beta}\right] - \frac{E_i}{R}\frac{1}{T}$$

Given an experimental data set (α, dα/dT and T), deconvolution of the kinetic curve (dα/dT - T) provides the FSi (T) approximations. The kinetic parameters Ei and Ai are estimated from the slope and the linear coefficient of the curve of ln $\left[\frac{FS_i}{f_i(\alpha)}\right]$ vs 1/T.

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Reabsorbable bioactive membranes based on bacterial cellulose and strontium apatite

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ARTICLE INFO

ABSTRACT

Guided bone regeneration (GBR) is a procedure for periodontal regeneration therapy. Its principle is to prevent an invasion of non-functional scar tissues and also to stimulate bone growth. Conventional materials of GBR membranes are generally, non-degradable polymers, which require a second surgery to remove the membrane after new bone generation. Therefore, reabsorbable membranes based on bacterial cellulose (BC) and strontium apatite (ApSr) - strontium is known to inhibit bone resorption and induce bone formation - were produced and evaluated aiming a future application as GBR membrane. BC was obtained from the cultivation of Komagataeibacter hansenii in Hestrin-Schramm medium. After purification, the membrane was submitted to oxidation by NaIO4 and functionalized with ApSr. Degradability tests were performed in phosphate buffered saline (PBS) and in simulated body fluid (SBF) at 37 °C. BC degradation products were quantified by HPLC. The bioactivity was verified by scanning electron microscopy (SEM). The results obtained from the analysis of the supernatant showed glucose as degradation product for both materials after 90 days. However, the degradation was higher for BC/ApSr than BC. Also the results showed that the degradation in PBS was higher than in SBF, probably because when the samples are immersed in SBF is induced a chemical precipitation with formation of hydroxyapatite, which indicate the bioactivity of the materials, as it was confirmed by SEM analysis. Therefore, the BC/ApSr is expected to be capable of initiating a bone formation process at the same time that it will probably be susceptible to degradation at physiological conditions.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Large-scale production of bacterial cellulose – Nexfill®

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ARTICLE INFO

ABSTRACT

Bacterial cellulose is a non-toxic, biocompatible biopolymer that has a significant impact on the development of biotechnological products. These characteristics make it a promising biomaterial in the health area. The production of biocellulose occurs through the metabolism of the bacterium *Gluconacetobacter xylinus* in static culture with optimal availability of carbon and nitrogen sources, and under controlled conditions of temperature, humidity and pH. In addition, stringent control during the production process ensures the high quality and productivity of large-scale biocellulose. Seven Indústria de Produtos Biotecnológicos Ltda. (Nexfill®) - invests in continuous research and improvement of the strain, aiming at a high performance in the production of biocellulose. Furthermore, it controls its processes to ensure the excellent quality of Nexfill® dressings. In order to evaluate the quality of the biocellulose, its thickness, weight and appearance of the membranes are taken into account. The company's current production capacity amounts to 6.000 membranes / month, which corresponds to 1.2 million cm^2 of biocellulose, and it can be increased according to market demand. When it comes to dressings for skin wound treatment, smooth or porous versions, this number is reflected in a monthly production of 24 thousand dressings 16x21cm or 48 thousand dressings 10x16cm or 96 thousand dressings 8x10cm. In addition to dressings, Seven developed the biocellulose hydrogel and invests in research and development for continuous improvement. The production of high quality bacterial cellulose on a large scale is only possible due to all the quality standards mentioned and maintained by the company.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Nexfill® biocellulose hydrogel - an innovation for skin wound treatment

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ARTICLE INFO

ABSTRACT

Bacterial cellulose membranes have biocompatibility *in vivo* and provide rapid healing when applied to skin wounds. The company Seven Indústria de Produtos Biotecnológicos Ltda. works with biocellulose since 1997 and currently produces this product on an industrial scale, dressing that aims to temporarily cover moist cutaneous wounds without infection, protecting the wound and accelerating the healing process. The company's commitment to developing new biocellulose based products has led to the development of a hydrogel aimed at improving the therapeutic response of biocellulose and facilitating application in different types of skin wounds. This study consisted of characterizing hydrogel and analyzing *in vitro* its effectiveness in healing. Hydrogel was characterized by rheological measurements from the flow curve, surface morphology and *in vitro* study of cell migration. The rheological results of the flow curve show that hydrogel has thixotropic characteristics, thus facilitating its application to the skin, as they become more fluid during application, facilitating scattering and then recovering the initial viscosity, preventing the hydrogel from flowing. In addition, the thixotropic hydrogels, since they do not undergo change of viscosity during storage, have a longer shelf-life. Through microscopic analysis, it was verified that hydrogel has on its surface porous structures and nanofibers of biocellulose, besides providing an acceleration in wound healing as suggested in the cell migration test, indicating the promising and potential application of this product for skin wound treatment.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Use of bacterial cellulose-based hydrogel in experimental Wounds of equines – preliminary results

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ARTICLE INFO

ABSTRACT

Wounds in horses are common and are subject of exuberant granulation tissue. Bacterial cellulose is a tissue repair material and it has been successfully applied to skin healing The objective of this study was to evaluate the healing of experimental wounds, treated or not with bacterial cellulose-based hydrogel gel 1% (BC-based hydrogel). Two wounds in the lumbar region were made in three horses, after sedation and local anesthesia. The daily treatment consisted on the application of physiological solution and, in the cranial wounds, BC-based hydrogel enough to cover the wound bed. The evaluation of the wounds was performed after the surgical procedure, and at 3, 7, 14 and 21 postoperative days, observing the presence of hemorrhage, clots, crusts, granulation tissue, epithelization and exudate, as well as photographic documentation, calculation of wound area and rate of contraction. During the first 7 days, bleeding, clots, exudate and crusts were observed. Granulation tissue and epithelization was observed after 3 and 7 days after surgery, respectively. Wounds were not fully healed by day 21, however, epithelization was evident in both groups. Although the wounds of the treated group were superior in the clinical evaluation, the areas of wounds in D21 were similar between the control and treated groups (1.2 and 1.5 cm^2, respectively), as did the contraction rate (64 and 51%, respectively). The continuity of the research, with microscopic evaluations and a greater number of animals, is necessary to elucidate the contribution or not of the BC in the healing of cutaneous wounds in this species.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Thermography of experimental wounds treated with bacterial cellulose-based hydrogel in equines – Preliminary Results

Daniela S. Denadai[1], Mariana Z. Guiati[1], Marcela da C. de Camargo[1], Juliana R. Peiró[1], Luiz Claudio N. Mendes[1], , Andréia B. Meneguin[2], Rafael M. Sábio[2], Hernane S. Barud[2], Alexandre L. de Andrade[1]Flávia de A. Lucas[1*]

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ARTICLE INFO

ABSTRACT

Introduction: Bacterial cellulose has been studied as a tissue repair material and its different variations have been successfully applied to skin healing. The objective of this study consist on evaluating the temperature kinetics during the cicatricial process of experimentally induced wounds, treated or not with BC-based hydrogel. Methodology: Three horses, adult and healthy, were used after sedation and local blockade. Two surgical wounds (4 cm^2) were made and skin and subcutaneous were removed, being a cranial (treatment) and caudal (control). Phenylbutazone (2.2 mg/Kg/IV/q24h) was administered for 3 days. Daily, the wounds were cleaned with physiological solution, and the cranial wounds were treated with BC-based hydrogel covering the wound bed. Thermographic images were obtained with Flir i60 camera, immediately after the surgical procedure (D0), and at 3 (D3), 7 (D7), 14 (D14) and 21 (D21) postoperative days. The temperatures of the center and the edges of the wounds were measured using the Flir Tools program. Results and discussion: Higher values of mean temperature of the center and edges were observed for treated wounds in D3 and D7relating to the control. Subsequently, a gradual decrease of the values was detected been the two groups similar in D14. Additionally, there was a temperature increase for both groups in D21, with similar values. Conclusion: Finally, the use of BC-based hydrogel in equine wounds is related to the increase of local perfusion and tissue metabolism, especially in the first 14 days of the healing process.

1º Encontro Brasileiro de Biocelulose

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In vivo study of wound healing of biocurative from bacterial cellulose with chitosan associated with ciprofloxacin in mice

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ARTICLE INFO

ABSTRACT

In research for dressings with most cost effective, the biopolymers gain prominence, especially bacterial cellulose and chitosan, which have proven efficacy in the treatment of lesions. Bacterial cellulose has high tensile strength, flexibility, water retention capacity and is non-toxic. In addition, its porosity allows the introduction and release of antimicrobial agents, drugs and other biofunctional materials. Chitosan, a biopolymer produced from the deacetylation of chitin, contains antibacterial effectiveness, emulsifying, and non - toxic, biocompatible and biodegradable properties. The present study aims at analyzing the cytotoxic, mutagenic and cicatricle characteristics of a biocurative produced by bacterial cellulose (BC) and chitosan (QTS) associated with a ciprofloxacin (BC/QTS/CIP) and comparing it to pure BC. All samples showed no cytotoxicity or mutagenicity. Through the in vivo tests, it was possible to analyze the capacity of maintenance of moisture in the interface curative / injury, acting as barrier for microorganisms, toxicity and absence of any sign of irritability in the lesion for both analyzed biocuratives. Regarding the area of healing, until the 7th day, the percentage of reduction of the lesion area was higher for the BC/QTS/CIP biocurative, however, on the 14th day, reepithelization was superior for the animals treated with BC and with formation of more mature tissue. On the 21st day, 100% healing of the injured area it observed in both cases. Finally, it concluded that the biocurative of pure BC, obtained with little difference superior results regarding the reduction of the lesion area, and both did not demonstrate cytotoxicity and mutagenicity.

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Morphological and chemistry analysis of bacterial cellulose membranes after pressure process

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ARTICLE INFO

ABSTRACT

Bacterial cellulose (BC), is a biopolymer applied in several fields. It has high water retention capacity, purity and crystallinity, is biocompatible, has fibers of nanometric size and has no lignin, pectin and hemicellulose in its structure, such as plant cellulose. However, little is known about possible changes that pressing treatment can cause in the morphological and chemical properties of CB. Therefore, the aim of this work is a chemical and morphological analysis of CB produced by *Komagataeibacter rhaeticus* strain after pressing process. The membranes were produced, purified, pressed with a hydraulic press according to central composite planning, evaluating 2 variables: pressing time 10, 20 and 30 seconds and pressing force of 1, 2 and 3 tons. The samples were analyzed by scanning electron microscopy (SEM) using the JEOL T-300 microscope operating at 2 kV and by infrared vibration spectroscopy (FTIR) with BRUKER 70 spectrometer from Bruker. SEM images of the treated samples compared the untreated samples showed more compacted fibers, less porous and aligned in the same direction according to the time and pressing force. However, the samples obtained by spectroscopy did not show chemical changes, which the CB presented characteristic bands. The results show that the use of the press can vary the structure morphology of CB.

1º Encontro Brasileiro de Biocelulose

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In-situ modification of biocellulose for drug delivery

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ABSTRACT

Bacterial cellulose (BC) has been intensively studied for many applications in the biomedical field including artificial blood vessels, tissue engineering, wound dressing, drug delivery, etc. However, plain BC films do not have biocidal activities neither are able to entrap, keep and control the release of antibiotics and proteins for therapeutic purposes. *In-situ* modification of BC scaffolds by adding exogenous molecules (*i.e.* polymers) to the culture media during BC synthesis allowed to drastically change the film properties. *In-situ* modification of BC with pectin allowed to efficiently encapsulate an antibiotic levofloxacin and human Serum Albumin. The Levo and HSA release profiles observed in the BC films in presence of HMP showed controlled and sustained molecular delivery along the time for 12 hours. Also, incorporation of alginate in BC network during bacterial growth showed a network able to entrap and properly doxorubicin. The release of the anticancer drug from BC-Alginate scaffold reduced the HT29 colon cancers cell growth in 67% after 48 h incubation. Structural analysis of the BC scaffolds modified with pectin and alginate by XRD, FTIR, DSC, and SEM demonstrated strong changes in the BC properties and interpenetrated matrices which allow to develop a class of BC films which displayed novel properties.

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Development of 3D-bioprinted pectin-biocellulose scaffolds for drug delivery

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ABSTRACT

The aim of the project is to develop personalized therapies using 3D-bioprinted dressing scaffolds for the treatment of ulcerated diabetic foot. The bioprinter developed in the Nanobiomaterials Laboratory (CINDEFI) is a 3D-cartesian printer commanded by an open source Arduino hardware and Marlin firmware. The 3D-bioprinter head was designed to extrude viscous materials using a syringe able to deposit 400 µm-strips over the plate with an air gap among filament of 750 µm. Hybrid pectins-cellulose biogels with different properties and compositions were assayed to be injected and making stable gel scaffolds on the printer plate. Biogels containing 20-25% (w/v) pectin plus 3-5% (w/v) nanocellulose were stable up 2.8 mm high using cylindrical and conical structural designs. The effect of amorphous (nanoparticles) and microcrystalline celluloses were compared in the film formation and stability. Biophysical analyses of scaffolds were performed by thermogravimetry (TGA), differential scanning calorimetry (DSC) and porosimetry.

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Development and characterization of biocuratives of bacterial cellulose with curcumine

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ARTICLE INFO

ABSTRACT

Bacterial cellulose is an abundant biopolymer, synthetized by the bacterium Komagataeirbacter rhaeticus, which shows characteristics and properties desirable for its use as a bandage in topical treatment of wounds. The aim of this study is to prepare and standardize bacterial cellulose bio-curative for the future incorporation of actives, such as curcumin, which has several pharmacological properties, like as antioxidant, antiprotozoal, antimicrobial, antiinflammatory and its application in wounds has shown an improvement in epithelial regeneration besides improving fibroblast proliferation and vascular density. For this to occur, bacterial cellulose membranes with curcumin will be produced and standardized and will be characterized by MEV, DSC and TG techniques, in order to demonstrate modifications in membrane structures with theincorporation of the compoundof interest in their matrix, to be produced a funcional bio-curative.

1º Encontro Brasileiro de Biocelulose

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Biocellulose nanofibers: a new generation of materials for application in the release of drugs

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ARTICLE INFO

ABSTRACT

The use of excipients having bioavailability to control the release of drugs in the body has been the subject of research, as they are able to decrease the daily amount of administration of the drug and its side effects, encouraging patients to adhere to its use. It was used as an alternative for the production of microcapsules, Bacterial Cellulose (CB), which has a nanometric structure, high surface area and fibers so intensely entangled, joined by hydrogen bonds, which when dried through the process of Spray drying imprison the drug within the matrix, increasing the release time thereof. Methodology: The production and purification of CB, Grinder defibrillation and spray drying were performed; were analyzed by Scanning Electron Microscopy (MEV) and by Liquid Absorption (AL). Results and discussion: Purification of CBs was sufficient to remove residues from the culture medium as well as bacterial residues, as well as defibrillation employing micro grinding in grinder was more adequate for the desired need. The results of AL revealed a pH-dependent behavior, with lower acid absorption, which should contribute to better control of the release when in the gastric environment.

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Development of filaments based on polymeric composites of phb (polyhydroxybutyrate) and bacterial cellulose obtained by addictive manufacturing for biomedical applications

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ARTICLE INFO

ABSTRACT

Additive manufacturing is an alternative method for producing new materials, and it has growned since the drop of the FDM (Fused Deposition Modeling) patent. FDM filaments are composed of thermoplastic polymers, but there is no a large variety of filaments available on the market. The main objective of this work has been the preparation and characterization of new filaments for 3D printing based on polymeric composites of PHB (polyhydroxybutyrate) and bacterial cellulose (BC). The composite filaments were produced using a simple screwthread extruder, varying the PHB-BC mass ratio. For the preparation of these composites, residues from the BC production were donated by Seven Biotecnologia Company. BC residue was powdered reduced using a Pulverizete until obtaining a micrometric powder that was mixed with PHB in different weight/weight proportions (1%; 0,7%; 0,5%; 0,3%; and 0,1%). The obtained filaments have been characterized by SEM images, infrared spectroscopy (FTIR), thermogravimetric (TG) analysis, and Differential Scanning Calorimetry (DSC), and in vitro tests. The average diameter of these new BC-PHB filaments were 1.75 mm and it could be a candidates for use in 3D printing in special for biomedical applicattions.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Development of filaments based on polymeric composites of phb (polyhydroxybutyrate) and bacterial cellulose obtained by addictive manufacturing for biomedical applications

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ABSTRACT

Additive manufacturing is an alternative method for producing new materials, and it has growned since the drop of the FDM (Fused Deposition Modeling) patent. FDM filaments are composed of thermoplastic polymers, but there is no a large variety of filaments available on the market. The main objective of this work has been the preparation and characterization of new filaments for 3D printing based on polymeric composites of PHB (polyhydroxybutyrate) and bacterial cellulose (BC). The composite filaments were produced using a simple screwthread extruder, varying the PHB-BC mass ratio. For the preparation of these composites, residues from the BC production were donated by Seven Biotecnologia Company. BC residue was powdered reduced using a Pulverizete until obtaining a micrometric powder that was mixed with PHB in different weight/weight proportions (1%; 0,7%; 0,5%; 0,3%; and 0,1%). The obtained filaments have been characterized by SEM images, infrared spectroscopy (FTIR), thermogravimetric (TG) analysis, and Differential Scanning Calorimetry (DSC), and in vitro tests. The average diameter of these new BC-PHB filaments were 1.75 mm and it could be a candidates for use in 3D printing in special for biomedical applicattions.

1º Encontro Brasileiro de Biocelulose

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BNC-based platforms for tumor growth models

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ARTICLE INFO

ABSTRACT

Many commercial tumor matrix biomaterials have uncontrollable cues that make molecular fine- tuned analysis difficult or near to impossible. Animal protein residues are particularly harmful. Critical questions such as the influence of growth factors in tumor development depend on highly defined matrix composition so they can satisfactorily rely on the target variables. The challenge is to start with a very pure bacterial nanocellulose (BNC) and establish a 3D culture environment that mimics tumor behavior. In particular, the vascular mimicry mechanism has recently been paid attention to due to possible relation with tumoral angiogenic process being included as a cancer hallmark. This work presents a bacterial nanocellulose-based 3D model platform for tumor growth studies and therapeutic strategies development. BNC membranes were produced by Komagataeibacter hansenii ATCC 23769 during a 4-days culture in a mannitol-based medium. Membranes were subsequently purified using caustic solution and extensive washing, fibers where oxidized and vascular promoters were chemically immobilized. Results show that vascular mimicry processes can be reliably modeled and controlled by adjusting nanofiber network density and topology. Our platform has been tested for a melanoma cell line with successful mimicry under in vitro culture controlled conditions. SK-MEL-28 from animal origin grew and spread forming vascular structures, analyzed by fluorescent microscopy. Resulting vessel network was characterized and quantified by image analysis. The developed BNC-IKVAV 3D hydrogel platform can provide a valuable tool to improve our understanding of microenvironmental cues in melanoma cancer progression, and their role in the vasculogenic mimicry process.

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In vitro study of osteo-1 on bacterial cellulose membrane surfaces modified using non-thermal plasma treatment

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ARTICLE INFO

ABSTRACT

Bacterial cellulose (BC), which has been used in a variety of medical applications, presents excellent malleability, hydrophilicity and biocompatibility. Due to these properties, this material is suitable for applications in tissue engineering as a temporary substitute for skin, as a support for drug interactions and for tissue growth. Success in using biodegradable polymers is determined by interactions between cells and the material employed, which are largely governed by the characteristics of the surface. The present work aims to evaluate the cellularization on a bacterial cellulose membrane (BCM) surface that has been modified using non-thermal plasma. OSTEO-1 will be cultivated in osteogenic differentiation medium in 48-well plates (5000 cells / mL) for 7, 14, and 21 days, in the absence and in the presence of BCM synthesized by *Acetobacter xylinum,* with or without modification of the membrane surface using non-thermal argon and oxygen plasma treatments. The culture medium will be exchanged every 72h, for alkaline phosphatase analysis. Cell adhesion and morphology will be analyzed by electron microscopy. Cell viability will be evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction method, using absorbance at a wavelength of 570 nm. Mineralization will be quantified by the Alizarin Red method, with measurement of absorbance at 405 nm. Cell counting will be performed with a Bio-Rad TC20 cell counter. We intend to develop a biopolymer derived from BC that offers improved cell adhesion and proliferation performance, which can be used in regenerative medicine and biomedical devices.

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Preparation of chemically modified cellulose hydrogel for bioprinting

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ARTICLE INFO

ABSTRACT

The tissue engineering study strategies for repairing and maintenance of alive tissues, and for such, the cells and the biomaterials are widely studied, once they form the systems responsible for the success of the tissue engineering. The behaviour of the cells depends on their interaction with the biomaterials for the formation of new tissues. Several technologies such as three dimensional printing and bioprinting have been arising for the development of new strategies to improve the repair and maintenance of tissues. The biomaterials used for these technologies are limited because they must show adequate properties for processing and also for keeping the cells viability. The cellulose chemically modified with TEMPO reagent is biocompatible and shows interesting properties for shaping by ionic complexation. In this vein, the proposal of this research project is the use of chemically modified cellulose hydrogel for incorporation of cells, followed by extrusion in form of fibers. The capability of fiber formation and maintenance of the cells viability will be the parameters to evaluate the efficiency of the material as a bionk for bioprinting application.

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Evaluation of the potential of cashew permeate as culture medium for bacterial cellulose production

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ARTICLE INFO

ABSTRACT

Bacterial cellulose(BC) is a biopolymer secreted by microorganisms that iscomposed by nanofibers with high crystallinity and purity,which provides a wide range of applications in several areas. In order to reduce the costrelated to BC production, agroindustrial sources rich in sugars and other nutrients have been widely studied as alternative culture media. This study evaluated the use of cashew permeate (CP), which is byproduct obtained in the process of extracting cashew pseudofruit fiber's aqueous extract rich in carotenoid, as a culture medium for BC synthesis. The fermentation was performed by static cultivation using three different CP concentrations (40, 50, and 100%; v/v) and a synthetic medium (Hestrinand Schramm; HS), for comparations purposes, using the *Komagataebacter xylinusATCC 53582*bacterial strain. The BCsproduced in HS and CP were characterized by thermogravimetry analysis(TGA), Fourier transforminfrared spectroscopy(FTIR) and X-ray diffraction(XRD). The characterizations results showed that BC from permeate presented typical behavior of bacterial cellulose, e.g. high purity and crystallinity and good mechanical properties.The BC'sproductionsin CP 40, 50 and 100% were 2.4, 2.8, and 3.50 g.L-1, respectively, which aregood production values.In fact,the BC'sproduction in CP, even at a lowerconcentration, was higher than the production reported by others fruit-based media.

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Study of microwave-assisted bacterial cellulose oxidation

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ARTICLE INFO

ABSTRACT

Bacterial cellulose (BC) is a biopolymer that has unique properties, which make it a promising materialfor application in several areas. The modification of the cellulose opens the possibility to introduce new desired properties. The dialdehyde cellulose derivative (DAC) can be produced employing periodate as an oxidizing agent. Factors such as time, temperature and periodate concentration directly influence the rate of oxidation. Microwave dielectric heating allows reactions that are not possible using conventional heating, with improved reaction yields and reduced reaction times. In addition, microwave heating is environmentally friendly and reduces operating costs. It is worth noting the lack of studies on the microwave-assisted oxidation reaction of bacterial cellulose. Therefore, it was intended in this work to optimize the oxidation process of BCby microwave-assistedheating and to compare with the process by conventional heating. BCfilms were obtained after the static fermentation of *Komagataeibacter hansenii*. To obtain the DAC, after being purified, the films were immersed in KCl/HCl buffer solution (pH 1) for 24 hours and oxidized with 1.5 g NaIO4: 1 g BC, where the reaction time and temperature were evaluated in the oxidation process. The aldehyde content (%) was determined by potentiometric titration.The results showed thatby conventional heating, to reach a content of 50% a reaction time of 360 min was required. While through microwave heating it was possible to decrease the reaction time to 30 min and achieve an aldehydecontentof 76%.

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Ce3+-doped calcium phosphates grown on biocellulose template for bone tissue engineering

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ARTICLE INFO

ABSTRACT

Biocellulose is a natural biopolymer produced by a variety of microorganisms belonging to different genera, such as *Gluconacetobacter*. Due to its remarkable physicochemical properties, besides its excellent biocompatibility, it has been demonstrated that biocellulose may be used as a template for the formation of calcium phosphates (CPs), which are the main synthetic bone grafts used. In order to improve antibacterial activity of CPs and to potentiate the differentiation, proliferation and mineralization of osteoblasts, an alternative has been the addition of dopant ions such as cerium ions (Ce3+). According to previous studies, it has been verified that those materials are notably promisor to applications in bone tissue engineering. In this context, we propose in this research the synthesis of Ce3+-doped CPs (CPsCe) (% Ce3+ = 5.00% m / m) by alternate soaking of biocellulose membranes, followed by calcination for 10 h at 500° C. The materials were characterized by XRD and SEM. EDS analysis indicated the presence of the Ce3+ ions. XRD patterns showed the presence of two phases: chlorapatite, (Ca10(PO4)6Cl2) and buchwaldite (NaCaPO4), once the precursors presented Na+ and Cl- ions, which were not completely removed during the intervals between the soaking cycles. The SEM images showed regions with *3D* porous body, with nanowires that are interconnected forming a resistant structure and suggesting the formation of mineral scaffolds, by growing CPs on biocellulose template.

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Proposal of new means of culture for production of bacterial cellulosis

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ARTICLE INFO

ABSTRACT

Due to its characteristics such as high purity, biocompatibility and biodegradability BC has been attracting the interest of researchers for its use in the medical and pharmaceutical area. However, because of the relative difficulty of obtaining on a large scale, this biopolymer it has been the subject of several researches, regarding the study of new methods and conditions of cultivation, mainly in relation to the carbon sources used. In this work were proposed two culture media with different carbon sources, synthetic medium 1, containing glucose and sucrose (MS1), synthetic medium 2 containing glucose, fructose and sucrose (MS2), to compare with the media described in the literature Zhou (Z), containing glucose, Yamanaka (Y) containing sucrose, Hestrin-Schramm (HS) containing glucose to BC produce. All media were inoculated with the bacterial strain *Gluconacetobacter hansenii* ATCC 23769 and maintained under static conditions for 7 days at 28 ° C. The experiment was carried in triplicate. After treatment to bacterial elimination and pH neutralization the BC were submitted to the complete dehydration to dry mass yield determination. Scanning Electron Microscopy analysis showed differences between the fibers the intertwining thickness and arrangement of fibers. The MS1 and MS2 dry mass yield presented higher values when compared with HS, Z, Y These results demonstrate an influence of the carbon sources present in the different culture media on the metabolic pathway for BC production.

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Development and characterization of microparticles reticulated with al3+ ions based on gellan gum nanocomposites reinforced with cellulose nanofibers

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ARTICLE INFO

ABSTRACT

Microparticles are multiparticulate systems of size ranging from 1 to 1000 μm with defined shape that have been used in the design of controlled drug release systems, since they allow the compartmentalization of the drugs and their protection against degradation exerted by external and internal factors.These systems are advantageous in relation to conventional release systems, since they allow the temporal and / or spatial control of the release, contributing to the increase of the therapeutic effect and reduction of side effects and toxic. The aim of thiswork was the development of microparticles based on gellan gum and reinforced with cellulose nanofibers (NFC) (3, 5 and 7 % m/v) as a potential strategy for controlled drug release. The microparticles were obtained through the ionotropic gelation process using Al3+ ions as crosslinking. The morphological analysis was performed by Scanning Electron Microscopy (SEM). The liquid absorption profile was evaluated in an Enslin device. Interactions between gellan and NFC was evaluated by Fourier Transform Infrared Spectrocospy (FTIR). The inotropic gelling method using Al3+ was efficient since it allowed the formation of spherical particles with reduced size (1210, 1060, 878 and 1210 μm). The NFC influenced the obtaining of the microparticles being used as reinforcement and it is possible to customize the size / shape of the particles. In the swelling, the addition of 3% of NFC was responsible for the lower absorption of liquids, which should also have a great impact on the control of release rates. The interaction between continuous phase (GG) and reinforcement (NFC) was confirmed by FTIR and occurred through hydrogen bonds. Therefore, it is concluded that the development and characterization of Al3+ crosslinked microparticles based on gellan nanocomposites reinforced with cellulose nanofibers has been successful and can be used in the design of controlled drug release systems.

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04 e 05 de junho de 2018

Cellulose productionusing as cultura medium soybean hulls hydrolyzate

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ARTICLE INFO

ABSTRACT

Soybean hulls are an abundant and low-cost lignocellulosic residual material that could be used as feedstock to obtain high added-value products. Due to its relatively low recalcitrance, soybean hulls canbe enzymatic hydrolyzed into a sugar-rich medium, without the need of any pretreatment process. Here, soybean hulls hydrolysate was evaluated as a source of sugars to maximize the production by bacterial cellulose, a high-value nanomaterial with remarkable properties and applications in the medical, electronic and automotive sectors. For that, soybean hulls were hydrolyzed by different commercial enzymatic cocktails and the hydrolysate with higher glucose concentration was used for bacterial cellulose production by *Gluconacetobacter hansenii*in comparison to the conventional Hestrin & Schramm culture medium. The nanocellulose films were fully characterized using SEM, XRD, FT-IR and TGA analyses. The use of hydrolysate supplemented with glucose resulted in 5.3 mg of bacterial cellulose, a value around 20% higher than the one achieved using the conventional medium. The films produced were similar in terms of thermal degradation behavior, with Tonset of about 300°C, and crystallinity (75%). FT-IR data confirmedthat the cellulose films were pure and composed of type I cellulose, showing that soybean hulls hydrolysate can be used as a potential feedstock for bacterial cellulose production.

1º Encontro Brasileiro de Biocelulose

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Cellulose productionusing as cultura medium soybean hulls hydrolyzate

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ARTICLE INFO

ABSTRACT

Soybean hulls are an abundant and low-cost lignocellulosic residual material that could be used as feedstock to obtain high added-value products. Due to its relatively low recalcitrance, soybean hulls canbe enzymatic hydrolyzed into a sugar-rich medium, without the need of any pretreatment process. Here, soybean hulls hydrolysate was evaluated as a source of sugars to maximize the production by bacterial cellulose, a high-value nanomaterial with remarkable properties and applications in the medical, electronic and automotive sectors. For that, soybean hulls were hydrolyzed by different commercial enzymatic cocktails and the hydrolysate with higher glucose concentration was used for bacterial cellulose production by *Gluconacetobacter hansenii*in comparison to the conventional Hestrin & Schramm culture medium. The nanocellulose films were fully characterized using SEM, XRD, FT-IR and TGA analyses. The use of hydrolysate supplemented with glucose resulted in 5.3 mg of bacterial cellulose, a value around 20% higher than the one achieved using the conventional medium. The films produced were similar in terms of thermal degradation behavior, with Tonset of about 300°C, and crystallinity (75%). FT-IR data confirmedthat the cellulose films were pure and composed of type I cellulose, showing that soybean hulls hydrolysate can be used as a potential feedstock for bacterial cellulose production.

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Evaluation of stability of bacterial cellulose in pbs and artificial saliva

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ARTICLE INFO

ABSTRACT

Bacterial cellulose is a polysaccharide widely used as biomaterial, having a high water retention capacity, excellent mechanical properties and being biocompatible. The objective of this study was to evaluate the stability of bacterial cellulose in PBS and in artificial saliva, which are simulated bodily media, aiming its use as biomaterial. The samples were placed in containers containing a solution of phosphate buffer, PBS (pH = 7.0) and in an artificial saliva solution (pH = 6.4). They were maintained at 37 ° C until 180 days had elapsed. Samples were analyzed every 30 days until 180 days. The samples were morphologically characterized (SEM) before and after the degradation, thermally (DSC and TGA), chemically (IR), besides mass loss and pH analysis of PBS and saliva. The mass loss analysis showed that the material was stable, and that in contact with the PBS it had a 3.7% decrease in mass, while in contact with the saliva, it was 9.3% in 180 days, demonstrating that saliva is a more aggressive bodily medium compared to PBS. In the pH analysis there was no significant variation with both media. The morphological analysis of the bacterial cellulose in contact with the saliva showed a degradation in the fibers, which did not occur in contact in PBS. Bacterial cellulose is a stable material when exposed in PBS, but it undergoes degradation when in artificial saliva.

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Insertion of metal phosphates in bacterial cellulose matrix for biomedical applications

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ARTICLE INFO

ABSTRACT

Bacterial cellulose (CB) is one of the most promising biopolymers because of its good mechanical properties, crystallinity, water retention capacity, interconnected 3D porous nanostructure, and excellent biocompatibility. These characteristics are advantageous in guided bone regeneration (GBR) due to the possibility of processing them into three-dimensional structures, in regeneration of many organs of the body, such as skin, cartilage, and others. Hydroxyapatite (HAp) is the main constituent of the inorganic components in the natural bone and can be combined with the HAp properties (biocompatibility, bioactivity and osteoconductivity) to promote several benefits to the bone tissue. Studies have shown that biomaterials based on tricalcium phosphates (TCP) with trace bone elements (Sr^{2+}, Zn^{2+}, Mn^{2+} and Mg^{2+}) may increase osteogenesis and neovascularization. Thus, the objective of this work was to develop BC scaffolds functionalized with five different metal phosphates, termed BC/$Ca_3(PO_4)_2$, BC/$Sr_3(PO_4)_2$, BC/$CaSr(PO_4)_2$, BC/$Zn_3(PO_4)_2$ and BC/$ZnCa(PO_4)_2$, aiming to induce bone growth for GBR application. CB membranes were biosynthesized by *Kumagataeibacter hansenii* and purified. Scaffolds were prepared from BC membranes by dipping sequentially in solutions of calcium chloride, strontium or zinc followed by phosphate solution. After functionalization, the samples were lyophilized for thermogravimetric analysis (TGA), Fourier transform infrared spectroscopy with attenuated reflectance accessory (FTIR/ATR) and scanning electron microscopy (SEM) characterization. TGA analyses showed that the amount of the mineral phase was around 72.5 to 83.3% with a total weight, further confirming the formation of apatite on the BC membrane. XRD patterns and FTIR spectroscopy, strongly suggest the doping ions in trace amount (Ca, Sr and Zn) influence at BC, it is enriched the biocomposite properties, that are essential for bone cells life. SEM micrographs have shown the deposition of biological apatite crystals which were affected by the ions inserted. Our results indicate the apatite formation on the BC membranes and trace amount of metal elements involved in bone formation, suggesting that these are potential biomaterials for use in biomedical applications, especially for guided bone regeneration.

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Combined use of cellulose biomembrane, photodynamic therapy and laser therapy in venous ulcer

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ARTICLE INFO

ABSTRACT

Venous ulcer is one of the most serious chronic venous diseases and is very common in the adult population, where 70-90% of cases are in the lower limbs, characterized by a discontinuous area of the epidermis and difficult to treat because of its complexity. METHODOLOGY: a randomized clinical study performed at the rehabilitation center of the Federal University of Triângulo Mineiro twice a week, by students of Physical Therapy and Physical Education. Among the techniques used, in the treatment of venous ulcers is photodynamic therapy (PDT) with blue LED and the administration of a topical photosensitizer (curcumin) in conjunction with laser treatment, celulose biomembrane application, ulcer hygiene, exercise and guidelines for home care. RESULTS: OAB participant, male, 74 years old, with ulcer in medial malleolus 3 years ago, initial area 13.91cm^2, current area 4.61cm^2. With respect to QV, improvement in the general health and vitality domains was observed, maintenance in the domains functional capacity, physical limitation and pain, and reduction of the mental health domain, which may be justified by the fact that the patient does not have a support network. DISCUSSION: the results showed na improvement in the healing process and pain improvement, which reflected the improvement of the results in the SF-36 questionnaire. CONCLUSION: it was observed that the proposed treatment presents promising results, bringing benefits to the health and quality of life of the participant.

1º Encontro Brasileiro de Biocelulose

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Bacterial Cellulose Membranes Modified with RGD Peptides for Skin Tissue Repair

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ARTICLE INFO

ABSTRACT

Bacterial cellulose (BC) is a biomaterial that has gained prominence in biomedical applications due to its structural and mechanical characteristics. Chemical modifications of BC have resulted in improvements in its characteristics without altering its main attributes, for example, in the mimetization of the local tissue environment and helping in the regeneration of tissues. The peptide sequence RGD is a main recognition site in several extracellular matrix protein that acts in the interaction between integrins and fibronectin promoting the cell adhesion. Therefore, immobilizing RGD peptides in BC can be attractive approach for new biomaterial development. Evaluate *in vitro* the potentiality of the use of membranes BC functionalized with peptides containing the RGD sequence for future applications in tissue regeneration processes. The modelled peptide (Acetil^{1521}WTGRGDSPA^{1529}NH$_2$) was synthesized by solid phase methodology, using a Rink BHA resin and Fmoc as protector of α-amino groups and t-Bu derivatives as side chains protectors of trifunctional amino acids. Hydrated BC was obtained from cultures of wild strains of *Gluconacetobacter xylinus*. The BC surfaces were characterized using Scanning electron microscopy (SEM) images obtained by a FEG JEOL 7500F microscope. X-Ray diffraction (XRD) patterns were performed on a Rigaku Rint 2000 Diffractometer (Rotating Anode). Fourier transform infrared (FTIR) spectra were obtained on a VERTEX70 BRUKER spectrometer using a diamond platinum ATR. SEM images showed that functionalized BC membranes showed no changes in surface morphology. XRD showed characteristic peaks of the cellulose. The FTIR spectrum of pure BC shows vibrating characteristics of cellulose. The functionalized membranes presented absorption bands of amide I and amide II, indicating the incorporation of the peptide in the membranes.

1º Encontro Brasileiro de Biocelulose

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Bioactive bacterial cellulose wound dressing

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ARTICLE INFO

ABSTRACT

Bacterial cellulose (BC) is already applied as commercial wound dressing. More recently, many studies based on BC wound dressings had focused at conferring new functionalities to this material. Thus, immobilization of proteolytic enzymes, such as papain, could add to the BC the ability to actively act in the removal of necrotic tissue, thus improving the healing process. The immobilization of enzymes via covalent bonding on cellulose offers an advantage, for example, increase the stability of the enzyme. Therefore, the objective of this work was to evaluate the biocompatibility and hemocompatibility of BC dressings containing immobilized papain. The BC was obtained by static fermentation of bacterial Komagataeibacter hansenii (ATCC 53582) in synthetic culture medium. Then, the BC was purified (K2CO3 at 80 °C for 1h) and oxidized (NaIO4 at 55 °C for 6 h). The immobilization of enzymes on oxidized BC was performed by immersing the film in a 2% (w/v) papain solution prepared in citrate-phosphate buffer pH 7 at 45 °C for 24 h. The bioactive dressing was characterized by indirect cytotoxicity test on human fibroblasts, which showed a cell viability greater than 89%, suggesting that the biomaterial is non-cytotoxic. Human blood contact tests showed that the immobilization of papain in BC decreased its ability to promote coagulation. However, both pure BC and the papain-BC dressing did not presented hemolytic activity, showing to be hemocompatible. Therefore, the biomaterial proposed in this work have potential to be applied as wound dressing on skin wounds.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Bacterial cellulose paper-based cell culture platform for biomedical applications

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ARTICLE INFO

ABSTRACT

Introduction: A variety of commercially available papers are used to produce cell culture platforms, which can be used to cultivate several cell lines. The surface of this material, however, does not provide adequate cell adhesion. In order to overcome this problem, paper surface modification becomes an attractive alternative, allowing change on its chemical structure and immobilization of biomolecules to its surface. Since paper is mostly made of cellulose, bacterial cellulose (BC) is a promising biomaterial to be used in this context, as it presents high biocompatibility, exhibits 3D architecture similar to the extracellular matrix and superior physical and mechanical properties compared to plant-derived cellulose. In this sense, the present work aims to obtain new and highly effective BC-based cell culture platforms. Methodology: BC membranes synthesized by Komagataeibacter rhaeticus in HS culture have been modified with the functional groups -NH 2 , -SH, -C n H 2n+1 and -C 6 H 5 by the use of four distinct silanes in acetone medium. The modified membranes will be immobilized with rhBMP-2 via adsorption in DMSO solution. Results and Discussion: The modified membranes will be analyzed by FTIR, XRD, RMN, TGA and SEM. Cultures of human fibroblasts and keratinocytes are going to be used to evaluate the membranes cell adhesion and proliferation, which will be analyzed by SEM in combination with quantitative analysis. Cell viability will be evaluated by the MTT assay. Conclusion: We intend to develop a BC-based platform that offers significantly enhanced cell adhesion and proliferation, which could be used in several biomedical devices and regenerative medicine.

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04 e 05 de junho de 2018

Development of multilayer biocomposite of bacterial cellulose and hyaluronic acid for therapeutic and cosmetic applications

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ARTICLE INFO

ABSTRACT

Bacterial cellulose (BC) and hyaluronic acid (HA) are biopolymers with excelente biological properties, but they present limitations in their physico-mechanics that limit their use in numerous clinical situations. The objective of this project is to develop and characterize multilayed biocomposite of BC / HA for therapeutic and cosmetic applications. For the production of hybrid membranes, sodium hyaluronate, MW = 1.1-1.7 × 106 Da, predispersed in water was added to the fermentation process at concentrations of 1, 5, 10 and 15%. After 10 days of fermentation the hybrid membrane floating on the surface of the culture medium was collected and immersed in an aqueous solution of 0.1 mol L-1 NaOH for one day for eliminate impurities such as bacteria and other interfering substances. In sequence the BC/HA multilayer pellicles were washed with deionized water several times to completely remove the alkali, and afterwards dried in stove at 40 C. The morphostructure of the hybrid membrane will be characterized by scanning electron microscopy (SEM). The confirmation of the incorporation of theHA will be evaluated by infrared spectroscopy with Fourier transform (FT-IR) and nuclear magnetic resonance spectroscopy (NMR). Termocalorimetry (TC) and surface energy evaluation will also be used to characterize the BC / HA multilayer biocomposite.

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Bacterial cellulose dressings containning silver nanoparticles loaded liquid crystals as potential strategy strategy for the treatment of complex wounds

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ARTICLE INFO

ABSTRACT

Bacterial cellulose (BC) and hyaluronic acid (HA) are biopolymers with excelente biological properties, but they present limitations in their physico-mechanics that limit their use in numerous clinical situations. The objective of this project is to develop and characterize multilayed biocomposite of BC / HA for therapeutic and cosmetic applications. For the production of hybrid membranes, sodium hyaluronate, MW = 1.1-1.7 × 106 Da, predispersed in water was added to the fermentation process at concentrations of 1, 5, 10 and 15%. After 10 days of fermentation the hybrid membrane floating on the surface of the culture medium was collected and immersed in an aqueous solution of 0.1 mol L-1 NaOH for one day for eliminate impurities such as bacteria and other interfering substances. In sequence the BC/HA multilayer pellicles were washed with deionized water several times to completely remove the alkali, and afterwards dried in stove at 40°C. The morphostructure of the hybrid membrane will be characterized by scanning electron microscopy (SEM). The confirmation of the incorporation of theHA will be evaluated by infrared spectroscopy with Fourier transform (FT-IR) and nuclear magnetic resonance spectroscopy (NMR). Termocalorimetry (TC) and surface energy evaluation will also be used to characterize the BC / HA multilayer biocomposite.

▷Cosmetics - Abstracts

1º Encontro Brasileiro de Biocelulose

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Peel-off facial mask containing propolis

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ARTICLE INFO

ABSTRACT

Peel-off facial masks are cosmetic products mainly obtained from film-forming vinyl resins, such as polyvinyl alcohol(PVOH). On the other hand, the associationof bacterial cellulose membranes(CB)with vinyl resins may improve the characteristics of regular peel-off marsksby conferringa net of nanometric channels. Propolis is a natural resin rich in phenolic compounds that have antioxidant, anti-inflammatory and antimicrobial activities. Therefore, the present work focused on the development and characterization of a facemaskrich in propolis bioactive compounds with peel-off technology. To obtain the facemask, CBfrom Nexfill Biotechnology wasprocessed(Turrax® Disperser)to obtain smaller particles. Next, CB was added with water, potassium sorbate and PVOH and allowed to stir until complete homogenization. Finally, the EPP-AF propolis extract was added and homogenized. Several compositionswere tested and the best formulationwas characterized byphysicochemical methodsand antimicrobial activity. As a result, a finely particulate sticky gel with burnt yellow colorcharacteristic, scatteringvalues of 42.80 cm^2/g(± 1,04), density 1.02 g/mL (± 0,02), pH 4.27 (± 0.03),flavonoidcontentof 0.30mg/g (± 0.01) and phenolic compounds of 1.59 mg/g (± 0.06) was obtained. The presence of Artepelin C, one of the main propolis compounds and related toseveral properties was determined.Additionally, animportant antimicrobial activity against different strains of *Staphylococcus aureus*(ATCC 25923 and 43300)and*Staphylococcus epidermidis* (ATCC 14990)was observed. Therefore,apeel-off facemask containing propolis bioactives and presenting antimicrobial activitywas successfully attained and could be employed in cosmetic facial treatments.

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04 e 05 de junho de 2018

▷Food - Abstracts

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Nanofibrillated bacterial cellulose and gelatin hidrolysate as baseto antioxidant films

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ARTICLE INFO

ABSTRACT

Edible polymers, natural compounds and nanotechnology are commonly associated in the research of high performance bioactive films, an alternative for the preservation and improvement of food quality and safety. Among the natural polymer bases applicable in the formulation of films, bacterial cellulose has unique properties with high potential in the food industry. In the present work, the effect of the amount of hydrolysateand type of plasticizer on the antioxidant activity of films based on nanofibrillated bacterial cellulose (NFBC) and gelatin hydrolysate from Nile tilapia skin(GHT) was evaluated. NFBCwas obtained by mechanical deconstruction of bacterial cellulose (BC) previously submitted to TEMPO -mediated oxidation. The antioxidant hydrolysatewas obtained by enzymatic hydrolysis of gelatin.Different concentrations of GHT andplasticizers in the formulation were evaluated. NFBC-GHTfilms were obtained by casting. And the antioxidant activity of the obtained film was analyzed by the DPPH method.The antioxidant activity in the film increased with increasing amount ofGHT.All unplasticized films presented a brittle appearance. In order to add resistance to films, two types of plasticizers (sorbitol andglycerol) were evaluated as to their influence on the activity of the obtained film.The use of sorbitol in the formulation increased the antioxidant activity of the film since the glycerol reduced the activity. In this way, it is concluded that the use of plasticizer is essential to obtain a more resistant NFBC-GHT film, highlighting the sorbitol against glycerol for promotinga more active film.

1º Encontro Brasileiro de Biocelulose

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Reinforcement of bacterial cellulose with recycled polystyrene for packaging applications

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ARTICLE INFO

ABSTRACT

Polystyrene (PS) is a synthetic polymer mainly produced in the expanded form (expanded polystyrene, EPS), commercially known as Styrofoam™, which is composed by only 2 wt% of PS. The mechanical and thermal recycling of EPS are not favored due to low density and release of toxic gases to the environment, respectively. The chemical recycling is an alternative approach for the EPS recycling because the dissolution in a solvent prevents the polymer degradation and there is no gas release into the environment. The use of d-limonene extracted from orange peel as a solvent is attractive since it comes from a renewable source and reduces about 95% of the original EPS volume. The resulting solution can be used for the production of self-cleaning and disposable papers, amphiphilic membranes and other applications as hydrophobic surfaces. In this way, different EPS solutions were prepared through the dissolution of EPS packages in d-limonene (10, 15 and 20 wt%), followed by the deposition onto bacterial cellulose (BC) surface using the airbrush technique. ATRFTIR indicated that the surface of BC was modified by the PS. The contact angle measurements showed an increase of the contact angle as the concentration of EPS solution increases, indicating na increase in the hydrophobicity of the samples due the presence of PS on the BC surface, as suggested by SEM results. Thermal analysis (TGA and DSC) indicated that there were no significantchanges in the thermal properties of bacterial cellulose.

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Edible films based on the combination of bacterial cellulose and pectin with fruit purees

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ARTICLE INFO

ABSTRACT

The industry primarily uses petroleum-derived materials as packaging, but this type of material accumulates in the environment. Thus, the development of packaging materials derived from natural sources is of great interest in the search for environmentally friendly products. Bacterial cellulose (BC) is a biopolymer synthesized by bacteria and presents interesting properties such as a nanostructured network, high crystallinity and high purity. Pectin, on the other hand, is one of the main components of plant cell walls, commonly used in the formation of cohesive and transparent films. The addition of fruit pulps to films is a way of providing them with color and flavor appeals. Moreover, fruits contain polysaccharides that contribute to film formation, as well as plasticizing sugars. In this study, films were prepared with different proportions of nanofibrillated BC (NFBC) and pectin, with or without fruit (mango or guava) purees, in order to evaluate the influence of the matrix composition and the presence of purees on film properties. The addition of purees increased the water vapor permeability (WVP), reduced tensile strength and modulus, and enhanced elongation. The replacement of pectin with NFBC made the films stronger, stiffer, more water resistant, and with decreased WVP. Pectin-based films may be applied as dissolving sachets, whereas cellulose-based films are the best choice for use as food wraps or coatings, which require better tensile properties and water resistance.

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▷In situ and ex situ modifications - Abstracts

1º Encontro Brasileiro de Biocelulose

04 e 05 de junho de 2018

Biocellulose gel with alginate on repair of excisional wounds in rats

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ARTICLE INFO

ABSTRACT

Wound healing is a natural biological response to tissue damage, which is knowed by a cascade of molecular events targeting tissue reconstruction. There are several therapies for wound healing. Bacterial cellulose membrane, commercially known as Nexfill® (Seven) have been shown as a promising biomaterial to treatment of wounds, providing a humid environment on the wound bed, improving scar formation and reducing pain on injured patients, as well it has a low cost and is easy to apply. In view of this, the relevant innovation is to evaluate the wound healing in rats with topical application of biocellulose gel with alginate. It was made two excisional wounds of 1.5 cm diameter on the dorsum of rats, which were divided into 4 groups: treated topically and 3x/week with Control gel, Nexfill, biocellulose gel with alginate (CB+AG) and Sham group (without treatment) to 0, 2, 7 and 14 days (n=5rats/follow-up days). The wounds were photographed on all follow-up days, the wound area was determinated by ImageJ software to calculate wound healing rate (WHR), which correspond to the formula [(initial area - final area) / initial area]. The Sham group showed superior WHR than Nexfill on the 2nd day. On the 7th day, Control gel was superior than Nexfill. On 14th day, all groups showed the wounds practically reepithelialized. Thus, Nexfill and CB+AG groups did not showed important reepithelialization by this macroscopic analysis. Other analysis will be carried out, however the results of these preliminary studies certainly relate to the texture/moisture of the gels.

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Komagataeibacter rhaeticus grown in sugarcane molasses-supplemented culture medium as a strategy for enhancing bacterial cellulose production

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ARTICLE INFO

ABSTRACT

Introduction: Although bacterial cellulose (BC) can be produced by a number of gram-negative bacteria, its production in standard culture medium in commercial quantities and economically competitive have been a challenge. In this sense, sugarcane molasses (SCM) has been proposed as a by-product from Brazilian fermentation industry that promotes costs reduction of culture media besides increase BC production. Herein, we evaluated BC production by *K. rhaeticus* in supplemented HS culture medium by adding SCM as alternative and cheaper carbon source (totally or partially). **Methodology:** BC membranes were prepared using seven distinct culture media and labeled F0 (standard HS medium), F1 (50 g/L of glucose), F2 (40 g/L of glucose plus 10 g/L of SCM), F3 (30 g/L of glucose plus 20 g/L of SCM), F4 (20 g/L of glucose plus 30 g/L of SCM), F5 (10 g/L of glucose plus 40 g/L of SCM) and F6 (50 g/L of SCM). **Results and discussion:** From FTIR, XRD and TGA results, great similarity among all membranes produced by distinct culture media were obtained. FEG-SEM analysis showed as higher SCM concentrations on culture media higher dense nanofibers network could be prepared. PeakForce (QNM-AFM) results displayed smoother and more flexible BC membranes as a function of the increasing of the SCM concentrations. **Conclusion:** The culture medium modification with an important by-product from Brazilian agroindustry appears as a viable alternative to reduce cost of BC production (of up to 20.06 %) besides increase the possibilities of industrial scale BC preparation.

1º Encontro Brasileiro de Biocelulose

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▷Multifunctional nanocomposites - Abstracts

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Bacterial cellulosemembrane with easy-cleaning properties

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ABSTRACT

Over the last decades, the design of high performance functional substrates with enhanced properties (eg. Easy-cleaning and Self-cleaning), has been the subject of intense research, offering promising improvements in a plethora of scientific and technological areas. The silica nanoparticles is promising nanomaterial for functional substrates owing to their remarkable properties and tunable surface chemistry. The ability to incorporate specific organic functional group onto their surface is another key parameter to engineer their properties in route to the target applications. Bacterial cellulose (BC) is secreted by a few strains of bacteria and consists of a cellulose nanofiber network with unique characteristics. Because of its excellent mechanical properties, outstanding biocompatibilities, and abilities to form porous structures, BC has been studied for a variety of applications in different fields, including the use as a biomaterial for scaffolds in tissue engineering. To extend itsapplications, BC is normally modified to enhance its properties. This work reports the preparation of Bacterial Cellulose Membrane (BCM) with Easy-Cleaning properties. Initially, sílica nanoparticles (SiO2) were prepared and functionalized with alkoxysilanes by co-condensation of TEOS (TetraEthOxySilane) with long alkyl chain and with a fluoroalkoxysilane. Subsequently, these nanoparticles were incorporated into BCM, following two different methodology: in situ and post-grafting. In situ functionalization of BCM, the culture medium composition was changed with the nanocomposites. On the other hand, in post-grafting functionalization, the BCM was modified after the BCM has been formed in culture.

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Production and characterization of all-cellulose films from bacterial cellulose

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ABSTRACT

Bacterial cellulose (BC) is a biomaterial with special properties and high technological potential to be exploited. In this sense, new routes that take advantage of BCproperties have been explored. All-cellulose composites are materials in which the matrix as well as the reinforcing agentare cellulose. These biocomposites could overcome the obstacle of a weak charge-matrix adhesion, usually foundin composites where matrix and reinforcement havedifferent nature. The objective of this work was to produce all-cellulose films from the deconstruction of BC, using nanofibrillatedBC (NFBC) as matrix and cellulose nanocrystals (BCNC) as reinforcement.The films were produced by the casting technique containingdifferent levels of BCNC (0-7.5% by weight),10g of cellulose NFBC, 5g of glycerol and distilled water (a solids content of 1g / 100ml). The suspensions werehomogenized in a high-speed blender (24,000 rpm, 15 min) andultrasonicated (60 Hz / 2 min). Then, the suspensionsweredeposited on a stainless-steel tray (20x30cm)witha final thickness of 0.100 mm and dried in an oven (50 °C / 48h).The films were characterized by thermogravimetric analysis, scanning electron microscopy, water vapor permeability (WVP) and percentage ofinsoluble matter. The films presented thermal profile characteristic of BCwith degradation peak around 350 °C. The microscopic images showed a homogeneous surface. The best WVPresult (2.2 g. Pa-1.h-1.m-1) was from the film containing5% of BCNCs. The percentage of insoluble matter was around 90% for all films, indicating high resistance to moisture. As conclusion,it is possible to produce all-cellulose films with good thermal stability, homogeneityand water vapor permeability.

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Bacterial cellulose nanofibers as reinforcement in the paper sheets formation

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ABSTRACT

Bacterial cellulose (BC) is a biopolymer that has a three-dimensional structure of nanometric fibers, giving it a large surface area, high absorption capacity, high water retention, elasticity and excellent mechanical strength. Suspensions of biocellulose fibers have been used as reinforcement in paper formation, since the amount of filler used can greatly interfere with the final properties of the composite. Thus, this study proposed to evaluate the influence of the presence of suspensions of bacterial cellulose fibers on the properties of paper sheets formed in laboratory with bleached eucalyptus pulp industrial pulp. The BC blankets were obtained by culturing the G.hansenii bacterium (ATCC 23769) in static culture medium, Hestrin Schramm (HS), with subsequent processing using the Ultraturrax and Grinder equipments, obtaining two suspensions called: bacterial nanofibers cellulose-Ultraturrax and nanofibers bacterial cellulose-grinder, characterized by the morphological pattern. The formation paper sheets with and without the addition of the suspensions of biocellulose, were obtained according to ABNT ISO 5269-1: 2006, using concentrations of 0,5%, 1,5% and 3% of fibers with subsequent determination of the physical properties of the leaves formed. The results demonstrated the presence of networks of intertwined microfibrils, without significant differences between them. With the results obtained in the physical tests performed on the leaves, it was possible to observe a decrease in the air permeability and capillary rise properties with the increase of the NBC concentration employed, together with a slight increase in the water absorption capacity, since the polymer has a highly porous system.

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Functional bionanomaterials based on bacterial cellulose

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ABSTRACT

Bacterial cellulose has been extensively used as an attractive environmentally friendly material for the preparation of multifunctional bionanomaterials. The biosynthesis of bacterial cellulose at the laboratory scale from bacterial cultures is an interesting and attractive biomimetic access to obtain cellulose with outstanding properties. This kind of cellulose is a natural nanomaterial with a high surface-to-volume ratio combined with good biocompatibility, high mechanical properties, and high crystallinity makes bacterial cellulose a potential material for applications in different fields. This work is a fruit of strong collaboration between UNESP, UNIARA and UPV/EHU. The aim of this work was the fabrication of hybrid inorganic/organic bionanomaterials based on laboratory made bacterial cellulose. This kind of materials linked together excellent properties of bacterial cellulose with the properties of inorganic nanoparticles such as optical, magnetic and electrical properties as well as chemical or biochemical activity. In addition, the functionalization of cellulose with inorganic materials opens new pathways for the fabrication of novel functional hybrid bionanomaterials with promising properties for a wide range of applications in different sectors. In this work, different pathways for fabrication of functional hybrid bionanomaterials with tunable properties based on bacterial cellulose modified with amphiphilic poly(ethylene oxide-b-propylene oxide-b-ethylene oxide) (EPE) block copolymer, sol-gel synthesized nanoparticles (titanium, vanadium and a mixture of both oxides) and functionalized iron oxide nanoparticles will be presented.

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Multifunctional flexible nanocomposites based on magnetic cobalt hexacyanoferrate nanoparticles immobilized on biocellulose nanofibers

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ABSTRACT

Introduction: Natural polymers templates capable to maneuver the growth and spatial distribution of functional nanoparticles have been pushing forward the development of a new generation of sustainable and versatile materials. Pure cellulose nanofibrils biosynthesized by bacteria naturally deliver a 3D interconnected network, lightweight, foldable and sustainable paper substrates or "nanopapers". Cellulose nanopaper is an exceptional biodegradable and biocompatible and high mechanical strength substrate with a native fibrous structure that can be easily applied as structure-directing host to produce nanosized materials with optical, electrical or magnetic properties. In this work, we investigated the preparation of magnetic nanopapers by using bacterial cellulose nanofibers to control the growth of molecule-based magnetic nanoparticles such as Prussian Blue analogues. **Methodology:** Magnetic Cobalt-Prussian Blue (CoHCEFe) nanoparticles were synthesized in situ by hydrothermal method through a diffusion-limited precipitation process onto bacterial cellulose nanofiber network labeled BC/CoHCEFe01, BC/CoHCEFe03 and BC/CoHCEFe05 nanocomposites. **Results and discussion:** Scanning Electron Microscopy and Atomic Force Microscopy clearly unveiled a homogeneous distribution of immobilized COHCEFe crystalline nanoparticles whose size decreases (from 94 to 70 nm) as a function of nanoparticle content (up 28 wt%). Magnetic Force Microscopy showed that these nanometric in size COHCEFe crystalline nanoparticles well-dispersed in BC fibers network respond on the magnetic field applied to the Magnetic Force Field-tip. **Conclusion:** This nano/nano association approach can provide functional flexible biocomposites for application in catalysis, adsorption of radionuclides, energy generation, data storage, biosensing, optical and magnetic nanopapers devices.