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

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ABSTRACT

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Bacterial cellulose: an ideal candidate for biomedical applications due to its biocompatibility, purity, crystallinity and stability, conferring unique characteristics to the final product. It is composed of a network of nanofibers that allow it to be used as a dressing, an ideal material for the treatment of burns since it can protect the wound and ensure that the surrounding tissue remains unaffected, also being able to work as a scaffold for the regeneration of tissue. However, this work is the first to show the effects of bacterial cellulose in a third-degree burn model.

Introduction

Burns are considered severe injuries occurring due to exposure of the skin to high-temperature physical or chemical 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 involves 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 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 is quickened and executed (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., 2012, 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 addition, deposition of the nanofibers in a 3D 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 bacterial cellulose a 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; Sarka, 2011).

In view of the abundance of characteristics presented, in addition to its macromolecular structure, this type of bacteria can also be 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 evidence that already proves 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 (C), where the animals were submitted to 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 studies were carried out according to the Brazilian Law 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, heated to a temperature of 700°C (Koura et al., 2006; Ko et al., Busuioiu 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; Camposello, et al., 2011). Immediately after injury the animals received 6.2 mg/kg 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 - Sao 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 loxidene variable, this membrane, still in its wet state, underwent a deposition process when they were subjected to a controlled spray of 20 ml of aqueous solution containing 4% lido dine. 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, Sao Carlos/SP (Anvisa registry - 80030810109) were used, plus gel composition, 50% bacterial gel cellulose gel, 0.15% nipa gan (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 (Zeiss, Guadix, Spain, 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 Nikon 5.0 imaging program. The fields were divided and inserted 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 analysis 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; Dan tas 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 analyses were performed in a blinded study by an experienced pathologist (Pessolato et al., 2011).

Statistical Analysis
For all the analyses 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 analyses 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).

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).

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 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 cellulse gel group (GelG).

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

Figure 3 - Percentage of collagen fibers. CG control group; MG bacterial cellulose membrane group and GelG bacterial cellulose 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 bacterial cellulose based cell used are found in the literature (Almeida et al., 2014; Fu et al., 2013; Aber 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 (Busuoc 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 other words, non-sterile or bioactive dressings, 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 dressings or gels. In addition, they report that the macromolecular structure of the biomaterial acted satisfactorily in protecting the wound 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 evaluation in the healing process when compared to the control.

Histologically, our results regarding the use of bacterial cellulose membrane for the first time demonstrated that the use of bacterial cellulose gel did not present satisfactory results, on the contrary, it has been 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 by the formation of a new structure, 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 the inflammatory reactions, thedressings are required with other formulations or even reduced application numbers in order for the evaluation to become more accurate.

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References