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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 bi-refringent 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).

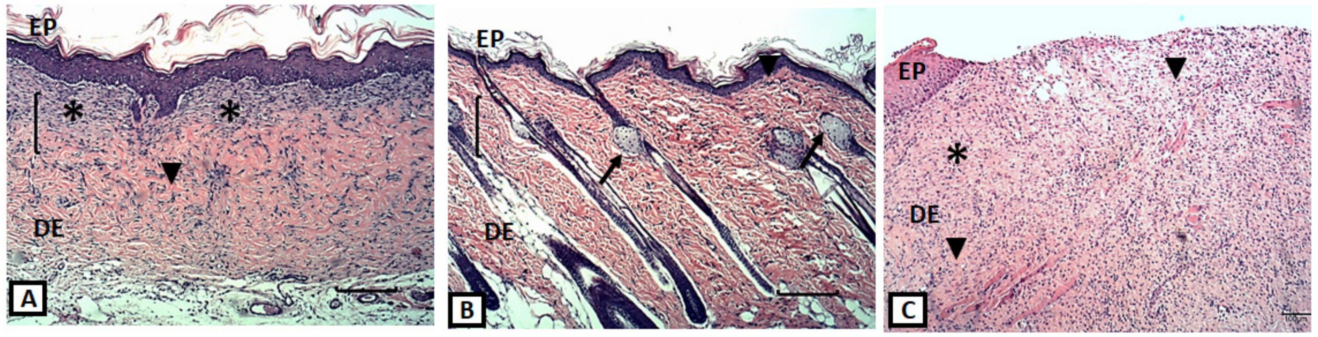

Figure 1- Representative photomicrographs of experimental groups stained with hematoxylin and eosin. (EP) epidermis, (DE) dermis, (*) fibrosis, (black arrow) skin attachments, (▼) inflammatory infiltrate. A - control group (CG) representing the skin only with the lesion, B - bacterial cellulose membrane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

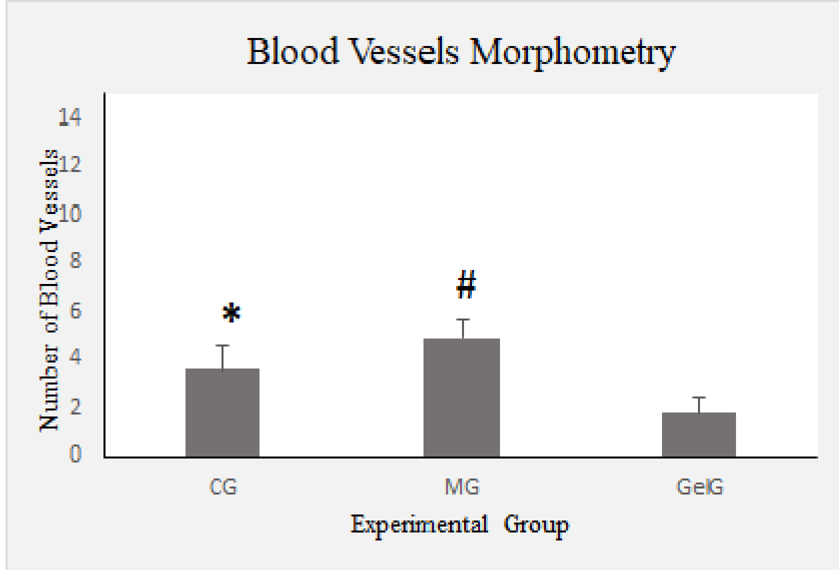

Figure 2 - Number of blood vessels. CG control group; MG bacterial cellulose membrane group and GelG bacterial celulose gel group.

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.

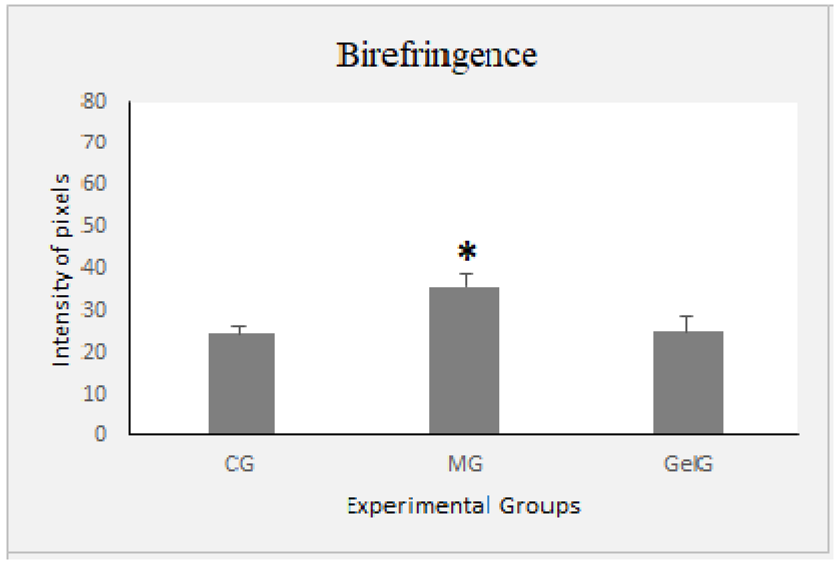

Figure 3 - Percentage of collagen fibers. CG control group; MG bacterial cellulose membrane group and GelG bacterial celulose gel group.

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.

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References

1. Turatti Pessolato AG, Martins DdS, Ambrosio CE, Furlanetto Mancanares CA, de Carvalho AF. 2011. Propolis and amnion reepithelialise second-degree burns in rats. *Burns* 37: 1192-201

2. Knabl JS, Bauer W, Andel H, Schwendenwein I, Dado PF, et al. 1999. Progression of burn wound depth by systemical application of a vasoconstrictor: an experimental study with a new rabbit model. *Burns* 25: 715-21

3. Shevchenko RV, James SL, James SE. 2010. A review of tissue -engineered skin bioconstructs available for skin reconstruction. *Journal of the Royal Society Interface* 7: 229-58

4. Sun G, Zhang X, Shen Y-I, Sebastian R, Dickinson LE, et al. 2011. Dextran hydrogel scaffolds enhance angiogenic responses and promote complete skin regeneration during burn wound healing. *Proceedings of the National Academy of Sciences of the United States of America* 108: 20976-81

5. Schwacha MG, Thobe BM, Daniel T, Hubbard WJ. 2010. Impact of Thermal Injury on Wound Infiltration and the Dermal Inflammatory Response. *Journal of Surgical Research* 158: 112-20

6. Soto-Pantoja DR, Shih HB, Maxhimer JB, Cook KL, Ghosh A, et al. 2014. Thrombospondin-1 and CD47 signaling regulate healing of thermal injury in mice. *Matrix Biology* 37: 25-34

7. Baxter RM, Dai T, Kimball J, Wang E, Hamblin MR, et al. 2013. Chitosan dressing promotes healing in third degree burns in mice: Gene expression analysis shows biphasic effects for rapid tissue regeneration and decreased fibrotic signaling. *Journal of Biomedical Materials Research Part A* 101: 340-48

8. Labus W, Kawecki M, Nowak M. 2012. The role of tissue engineering in the treatment of burn wounds. *Pol Przegl Chir* 84: 167-71

9. Avila HM, Schwarz S, Feldmann E-M, Mantas A, von Bomhard A, et al. 2014. Biocompatibility evaluation of densified bacterial nanocellulose hydrogel as an implant material for auricular cartilage regeneration. *Applied Microbiology and Biotechnology* 98: 7423-35

10. Abeer MM, Amin MCIM, Martin C. 2014. A review of bacterial cellulose-based drug delivery systems: their biochemistry, current approaches and future prospects. *Journal of Pharmacy and Pharmacology* 66: 1047-61

11. Cheng KC, Catchmark JM, Demirci A. 2009. Enhanced production of bacterial cellulose by using a biofilm reactor and its material property analysis. *J Biol Eng* 3: 12

12. Hu W, Chen S, Yang J, Li Z, Wang H. 2014. Functionalized bacterial cellulose derivatives and nanocomposites. *Carbohydrate Polymers* 101: 1043-60

13. Almeida IF, Pereira T, Silva NHCS, Gomes FP, Silvestre AJD, et al. 2014. Bacterial cellulose membranes as drug delivery systems: An in vivo skin compatibility study. *European Journal of Pharmaceutics and Biopharmaceutics* 86: 332-36

14. Saska S, Barud HS, Gaspar AM, Marchetto R, Ribeiro SJ, Messaddeq Y. 2011. Bacterial cellulose-hydroxyapatite nanocomposites for bone regeneration. *Int J Biomater* 2011: 175362

15. Trovatti E, Silva NHCS, Duarte IF, Rosado CF, Almeida IF, et al. 2011. Biocellulose Membranes as Supports for Dermal Release of Lidocaine. *Biomacromolecules* 12: 4162-68

16. Benlier E, Eskiocak S, Puyan FO, Kandulu H, Unal Y, et al. 2011. Fucoidin, a neutrophil rolling inhibitor, reduces damage in a rat electrical burn injury model. *Burns* 37: 1216-21

17. Brassolatti P, Bossini PS, Oliveira MC, Kido HW, Tim CR, et al. 2016. Comparative effects of two different doses of low-level laser therapy on wound healing third-degree burns in rats. *Microsc Res Tech* 79: 313-20

18. Muniz Renno AC, Toma RL, Feitosa SM, Fernandes K, Bossini PS, et al. 2011. Comparative Effects of Low-Intensity Pulsed Ultrasound and Low-Level Laser Therapy on Injured Skeletal Muscle. *Photomedicine and Laser Surgery* 29: 5-10

19. Bossini PS, Rennó ACM, Ribeiro DA, Fangel R, Ribeiro AC, et al. 2012. Low level laser therapy (830 nm) improves bone repair in osteoporotic rats: Similar outcomes at two different dosages. *Experimental Gerontology* 47: 136-42

20. Brassolatti P, Kido HW, Gabbai-Armelin PR, Bossini PS, Almeida -Lopes L, et al. 2015. Bacterial Cellulose Membranes as Applied Natural Dressing in the Treatment of Full-thickness Burns in Rats. *Tissue Engineering Part A* 21: S135-S35

21. Nunez SC, Franca CM, Teixeira Silva DF, Calvo Nogueira GE, Prates RA, Ribeiro MS. 2013. The influence of red laser irradiation timeline on burn healing in rats. *Lasers in Medical Science* 28: 633-41

22. Bossini PS, Fangel R, Habenschus RM, Renno AC, Benze B, et al. 2009. Low-level laser therapy (670 nm) on viability of random skin flap in rats. *Lasers in Medical Science* 24: 209-13

23. Bossini PS, Fangel R, Ribeiro AC, De Assis Lahoz M, Crovace M, et al. 2014. Biosilicate and low level laser therapy improve bone repair in osteoporotic rats. *Journal of Tissue Engineering and Regenerative Medicine* 8: 431-32

24. Colombo F, Neto AdAPV, Sousa APCd, Marchionni AMT, Pinheiro ALB, Reis SRdA. 2013. Effect of Low-Level Laser Therapy (660 nm) on Angiogenesis in Wound Healing: A Immunohistochemical Study in a Rodent Model. *Brazilian Dental Journal* 24: 308-12

25. Tim CR, Zambone Pinto KN, Orsini Rossi BR, Fernandes K, Matsumoto MA, et al. 2014. Low-level laser therapy enhances the expression of osteogenic factors during bone repair in rats. *Lasers in Medical Science* 29: 147-56

26. Lu Q, Tang L, Lin F, Wang S, Chen Y, et al. 2014. Preparation and characterization of cellulose nanocrystals via ultrasonication-assisted FeCl3-catalyzed hydrolysis. *Cellulose* 21: 3497-506

27. Fu L, Zhang J, Yang G. 2013. Present status and applications of bacterial cellulose-based materials for skin tissue repair. *Carbohydrate Polymers* 92: 1432-42

28. Czaja WK, Young DJ, Kawecki M, Brown RM, Jr. 2007. The future prospects of microbial cellulose in biomedical applications. *Biomacromolecules* 8: 1-12

29. Busuioc CJ, Mogosanu GD, Popescu FC, Lascar I, Parvanescu H, Mogoanta L. 2013. Phases of the cutaneous angiogenesis process in experimental third-degree skin burns: histological and immunohistochemical study. *Romanian Journal of Morphology and Embryology* 54: 163-71

30. Fu L, Zhang Y, Li C, Wu Z, Zhuo Q, et al. 2012. Skin tissue repair materials from bacterial cellulose by a multilayer fermentation method. *Journal of Materials Chemistry* 22: 12349-57

31. Portal O, Clark WA, Levinson DJ. 2009. Microbial Cellulose Wound Dressing in the Treatment of Nonhealing Lower Extremity Ulcers. *Wounds-a Compendium of Clinical Research and Practice* 21: 1-3

32. Reinke JM, Sorg H. 2012. Wound Repair and Regeneration. *European Surgical Research* 49: 35-43

33. Blomme EAG, Chinn KS, Hardy MM, Casler JJ, Kim SH, et al. 2003. Selective cyclooxygenase-2 inhibition does not affect the healing of cutaneous full-thickness incisional wounds in SKH-1 mice. *British Journal of Dermatology* 148: 211-23

34. Futagami A, Ishizaki M, Fukuda Y, Kawana S, Yamanaka N. 2002. Wound healing involves induction of cyclooxygenase-2 expression in rat skin. *Laboratory Investigation* 82: 1503-13

35. Goldyne ME. 2000. Cyclooxygenase isoforms in human skin. *Prostaglandins & Other Lipid Mediators* 63: 15-23

36. Brassolatti P, Kido HW, Bossini PS, Gabbai-Armelin PR, Otterço AN, Almeida-Lopes L, Zanardi LM, Napolitano MA, Forato LA, Araújo-Moreira FM, Parizotto NA (2018) Bacterial cellulose membrane used as biological dressings on third-degree burns in rats. Biomed Mater Eng 29(1):29-42