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EFFECTIVENESS OF THE HYDROGEL DRESSING CROSSLINKED AND STERILIZED BY GAMMA RADIATION

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ABSTRACT

Chronic wounds of difficult cicatrization are more incidents with an increased in life expectancy. The hydrogel with silver nanoparticles developed at the Nuclear Energetic Research Institute, crosslinked and sterilized by Gamma radiation, is inexpensive and has a simple manufacturing process that seems to be an alternative to the treatment of injuries. This experimental study compared the healing process of second intention of skin wounds of 4cm² on the back of 69 Wistar rats, considering the effectiveness of hydrogel dressings with silver nanoparticles. The animals were distributed in three groups that received treatment with hydrogel dressing, hydrogel with 22ppm of silver nanoparticles and hydrogel with 44ppm of silver nanoparticles, and the dressings were performed every 48 hours. The project was approved by the Animal Experiments Committee. Hydrogel dressings are transparent and allow accurate visualization of the center of the lesion. The part of the dressing in contact with the wound kept the medium moist, promoting interaction with the fluid of the lesion, besides adsorbing the moisture produced. The dressing replacement didn't cause discomfort or pain, since the animals were manually contained during the procedure, and the cover is easy to remove without causing trauma to the healing tissue. Wounds remained free of fibrin formation and necrosis, and serous exudation of lesions was scarce. At 21 days all the lesions were healed showing that dressings weren't negative to the second intention healing process.

1. INTRODUCTION

The increase in population life expectancy is a reality lived worlfwide, specifically in developing countries, as in Brazil. In contrast to aging we have the incidence of chronic and degenerative diseases, and linked to this process the increase in the number of skin lesions, known as wounds[1].

Wound is any lesion that causes discontinuity in the epithelial tissue. Affect any person, at all ages, and may present several origins[2]. There are many ways to classify a wound, but the development time is chronic or acute, and the great challenge for health professionals is the ideal treatment that stimulates tissue healing, either acute or chronic[3].

The evaluation and clinical follow-up of patients with wounds as well as injuries is essential for the success of the treatment. In performing the dressing, the extent of the lesion should be monitored (surface area, depth and the presence of tunnels and detachments), the color of the tissue (granulation, fibrin, necrosis), adjacent tissue (coloration, hydration and circulation), secretion (quantity and appearance), odor (intensity and characteristics), and pain (type, intensity, moment)[2, 4].

Numerous factors are involved in the healing process, and go beyond the factors involved and inherent in the healing phases, such as neovascularization, fibroblasts, collagen and elastin. In this context, the choice of the dressing has a unique role for treatment sucess. And the choice of the ideal dressing is not an easy decision, considering the vast supply market for wound dressings [2, 5].

Several dressings currently exist in the market and choosing the ideal treatment for a wound is dependent on the type of injury and of the clinical characteristics presented in the tissue, and during the treatment of a lesion hardly the same dressing will be maintained from the beginning to the end. The ideal coverage should offer more protection and less risk for infections and promote good healing in humid environment. It is also desirable that the dressing be easy to apply and removed, have a satisfactory cost-benefit ratio, present pain control, and provide improved self-esteem and self-image of patients [4].

The hydrogel with silver nanoparticle manufactured at the Insitute the Energetic Nuclear Research is inexpensive (accessible to any health care user) and has a simple manufacturing process that appears to be an alternative to the treatment of acute and chronic injuries. Pre-clinical studies of hydrogel dressings with nanoparticles are necessary for the standardization of dressing as well as follow-up of clinical trials in various conditions in which the use of silver may be indicated.

2. MATERIALS AND METHODS

It is an experimental, exploratory, descriptive and quantitative preclinical study using pure hydrogel dressings, hydrogel dressings with 22ppm and 44ppm of nanoparticules in the treatment of superficial wounds in *Rattus norvegicus*, Wistar lineage. Eighty-nine male animals, young adults, with mean age between 90 and 120 days were used. The research was conducted at the College Antônio Carlos Porto (FAPAC) in Porto Nacional, Tocantins and Histology Laboratory of the Faculty of Medicine and at the Biological Sciences Institute of the University of São Paulo (USP).

During the setting period and during the experiment the animals were able to control the light and dark cycle, temperature control (22 ± 2ºC), air conditioning, humidity control (50-70%) and ambient pressure. The animals were individually housed in the suspended cages with acrylic and plastic bottoms and sterile bedding [6], the flakes were obtained from Pinus elliotti wood, previously sterilized in the vivarium, and the product complied with the MTC-CONCEA

Legislation RN 33. They received filtered water and food ad libitum[7], being fed with special ration rodent feed for the Nuvilab® brand. In accordance with environmental enrichment legislation, chopped paper strips and cardboard tubes were placed in the cages [7].

The project was submitted and approved by the Committee on Ethics in Animal Research of the College President Antônio Carlos - Itpac Porto under number 006/2017, taking into account all aspects of the resolutions, guidelines and regulations in force.

The animals were divided into three groups, according to the applied dressing treatment, being: the control group (using hydrogel dressing); Treatment group 1 (used hydrogel dressing with 22ppm nanoparticle); and treatment group 2 (using hydrogel dressing with 44ppm of nanoparticle). On the day of the experiment the animals were anesthetized and surgical asepsis was carried out following the marking of the surgical area and after removal of 20mm x 20mm of superficial skin surface of the animal's back, with removal of the skin tissue to the muscular fascia (Figure 1). The withdrawn area was kept exposed to the healing dressing by second intention [8, 9].

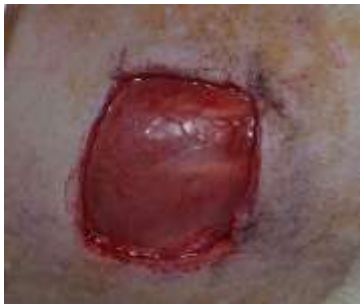

Figure 1: Removal of the tissue to the muscular fascia for healing in a second intention.

Each wound was treated immediately after the surgical procedure and every two days with a solution salina (NaCl 0.9%) and the dressing was applied, moistened with distilled water. The hydrogel and hydrogel dressings with silver were covered with gauze and fixed to the animal with the aid of micropore tape [9]. To maintain fixation of the micropore and the containment of the dressing in the animal was used fixation with elastic of 4cm.

The animals were monitored daily for suffering or stress through clinical follow-up on appearance, behavior change, self-mutilation and vocalization [6, 7]. The evaluation of the weight of the animals had as objective to verify possible developmental compromises caused by the use of the dressing and also the food consumption of the animals was controlled by weighing in grams of the available ration *ad libidum.*

The lesion of the animals was evaluated in an appropriate table for measurements and dressing. The size of the lesion extension was measured through the aid of Digimess digital caliper and the lesions were measured in the area of greatest length and width. All lesions were photographed with Nikon model d3200 camera fixed on a tripod at the standard distance of 15cm from the lesion to maintain the standardization of photographic records. The photos were performed without flash, in a lighted place and with the animal contained manually.

The macroscopic assessment of the wound was performed by observing the healing tissue during the dressing. The tissues were classified as percentage in crust, necrosis, fibrin, granulation and epithelization. The amount of exudate from the wound was measured as scarce, moderate or intense. The exudate was also classified was serous, blood or purulent. As to the

edges of the lesion were evaluated and classified as intact, edemaciate, hyperemidas, macerated, desquamated, resected, keratosis or callosity. And the area adjacent to the wound was followed for swelling and hyperemia.

The hydrogel and hydrogel with silver nanoparticles produced by IPEN are synthesized from gamma radiation, without the use of catalysts or other reagents. It is possible to reticulate and sterilize with Co60 gamma radiation at the same time, guaranteeing a simple dressing with antimicrobial action, since the use of radiation is effective for the synthesis of nanosilver [9].

The hydrogel consists of a polymer blend of 8% poly (N-vinyl-2-pyrrolidone) (PVP), 1.5% polyethylene glycol (PEG), 1% agar and 89.5% reverse osmosis water forming a three-dimensional network composed of polymers crosslinked by ionizing irradiation [10, 11]. In the dressings composed of silver ionic silver (Ag +) is added in the concentrations of 22ppm and 44ppm, after which they are transformed into nanoparticles. All dressings are sterilized at 25kGy. It is a low cost, sterile dressing with antimicrobial action of simple formulation compared to commercial formulations in the market.

3. RESULTS AND DISCUSSIONS

After the 30 days of the experiment, all the animals presented normal appearance, absent self-mutilation and/or vocalization.

Evaluating the pain and stress of the animals is important not only for having undergone the surgical procedure of research, but also for being tested a product of dressing that among the particularities proposed for healing promotes a humid environment with decreased pain. During the whole experiment, food consumption was monitored, with no change in food grams between groups. It is emphasized that the dressing was changed in the animal without sedation, only with manual containment of the same.

In this study, dressing changes occurred every 48 hours, mainly in the inflammatory period, where there is greater vasodilation and tissue edema occur, a period that demands greater moisture to the lesion. The hydrogel dressings used are transparent and allow the exact visualization of the center of the lesion (Figure 2), ensuring that it is well distributed with free edges.

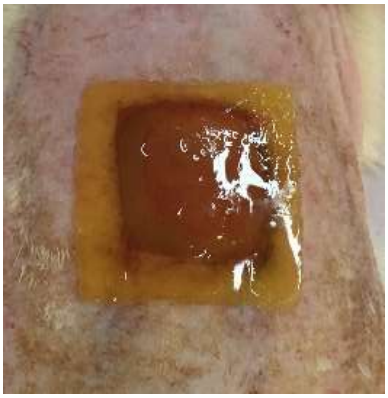

Figure 2: Transparency of the curative applied on the lesion with free edges.

It was observed that the part of the dressing that was in contact with the wound kept the medium moist, promoting interaction with the fluid of the lesion, and being easy to touch without

causing trauma to the tissue. However, the free margin of the dressing that remained in contact with the skin of the animal resected considerably losing water to the external environment (Figure 3).

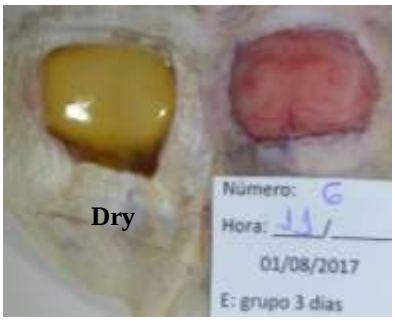

Figure 3: Removal of the curative without trauma and margins of the dressing in contact with the resected whole skin.

In the dressing changes when the covering had moved from the center of the lesion, the penso cover adhered to the wound causing bleeding of the tissue when removed, even with intense hydration of the adhered area (Figure 4).

Figure 4: Dislocated dressing from the central area of the lesion kept part of the wound in contact with the gauze and part in contact with the hydrogel.

The longer it is possible to keep the dressing on a wound without the need for exchange, the greater the comfort for the patient and the less stress the healthcare team involved in the treatment [12]. Clinical study using hydrogel membranes with silver nanoparticles observed efficiency of the cover as it did not adhere to the wound, promoted the moist environment for the body enzymes to perform the action of cellular degradation, in addition to reducing wound odor as well as the size and quantify of exudate [13]. The hydrogel dressing of this study proved to be adequate for healing because it does not adhere to the wound site, allowing the absence of new traumas in the tissue, as it is free of adherence in the wound bed. The removal is easily performed by reducing and / or absenting painful stimuli, a fact proven by the exchange of the dressing only with manual containment of the animals.

In the follow-up of the wounds during the thirty days of the experiment, the lesions remained free from the formation of fibrin and necrosis. When the dressing was displaced from the center of the lesion, the penso adhered to the wound, traumatizing the tissue at the moment of removal. The exudate of the lesions were always scarce not exceeding the coverage area. The dressing showed to be able to adsorb the moisture produced, and the exudate was always serous, and there was no clinical worsening of the lesion with the hydrogel coverage. Wet cicatrization is faster and avoids the formation of necrosis. The ideal dressing should promote a moist environment in the lesion and adjacent region, allow gas exchange, absorb the exudate without causing maceration at the margins of the lesion, protect against microorganisms, preventing

infection, stimulate tissue growth, provide mechanical protection for trauma, help reduce pain, and allow for exchange whitout trauma, be sterile and low cost [1].

The dressing changes were performed every 48 hours, however it was observed that in the proliferation and remodeling phase the coverage could be maintained for a longer time. It was chose to maintain the standard of the exchange time due to the movement of the animals that sometimes ended up displacing the cover of the center of the lesion. It was not possible to increase the perillary margin area of the cover as it ended up drying and adhering to the coat of the animal, causing discomfort at the moment of removal. The edges of the wounds remained intact, absent from dryness, keratosis, detachment or any other complication, as the adjacent area did not evolve with edema and / or hyperemia that could indicate reaction to the covering or process infectious disease in evolution.

There is a vast market for wound care dressings, but to choose the ideal dressing it is necessary to evaluate the cause of the wound, the location and size of the wound, the amount of exudate, the wound bed and the potential for contamination; in addition to considering factors intrinsic to the human being, such as age and associated comorbidities [3, 5, 14]. And it is associated to these questions the requirements for an ideal dressing: non adherence to the wound bed, reduced number of exchanges, absorption of exudate, protection of the lesion, influence in the absence or decrease of pain, stimulation of the scar tissue and especially low cost and accessible to the entire population [4, 5, 15].

4. CONCLUSIONS

The hydrogel curative produced at IPEN has the characteristics of an ideal dressing, favored the humid environment, promoted the restoration of tissues and allowed the absence of traumas and pain. And the dressing is low cost being an excellent opportunity.

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REFERENCES

1. ALCANTARA, M. T. S. *Hidrogéis Poliméricos com Nanopartículas de Prata para Aplicações Médicas.* Tese (Doutorado em Ciências na Área de Tecnologia Nuclear – Materiais) Instituto de Pesquisa em Energia Nuclear, São Paulo, Brazil, 166p. (2013).
2. CARDOSO, L.; USERO, R.; CATANI, S. M. M.; BARBO, M. L. P.; ESPOSITO, A. R.; DUEK, E. A. R. "*Implante de membranas de PLLA/Trietil-Citrato como alternativa no tratamento de feridas cutâneas".* Polímeros [online]. **Vol 23**, n. 6, p. 798-806, (2013).
3. CARDOSO, T. R. *Aplicabilidade de Curativos a base de hidrogel com nanopartículas de prata em lesão por pressão*. Dissertação (Mestre em Ciências na Área de Tecnologia Nuclear – Materiais) Instituto de Pesquisas Energéticas e Nucleares, São Paulo, Brazil, 67p (2017).
4. FARINA JUNIOR, J. A.; COLTRO, P. S.; OLIVEIRA, T. S.; CORREA, F. B.; CASTRO, J. C. D. "Curativos de prata iônica como substitutos da sulfadiazina para feridas de queimaduras profundas: relato de caso". *Rev Bras Queimaduras*. **Vol 16**, n. 1, pp 53-7 (2017).

5. GEOVANINI, T. *Tratado de Feridas e Curativos - Enfoque Multiprofissional*. Rideel, São Paulo, Brazil (2014).
6. GIRARD, R. C. G. *Comportamento de Matrizes de colágenos utilizadas no tratamento de feridas planas induzidas em pele de ratos*. Dissertação (Mestrado em Bioengenharias) Instituto de Química de São Carlos. Universidade de São Paulo, Brazil 101p. (2005).
7. IRION, G. L. *Feridas: Novas Abordagens, Manejo Clinico E Atlas Em Cores*. Guanabara Koogan, Rio de Janeiro, Brazil (2012).
8. KAMOUN, E. A.; KENAWY, E. S.; CHEN, X. "A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings". *Journal of Advanced Research,* **8**, pp. 217–233 (2017)
9. KIERSZENBAUM, A. L.; TRES, L. L. *Hitologia e Biologia Celular: uma introdução à patologia*. Elsevier, Rio de Janeiro, Brazil (2012).
10. KUMAR, V.; ABBAS, A. K.; ASTER, C. J. *Robbins Patologia Básica*. Elsevier, Rio de Janeiro, Brazil (2013).
11. MALAGUTTI, W. *Feridas: Conceitos e Atualidades*. Martinari, (2015).
12. NATIONAL INSTITUTE OF HEALTH (NIH). *Guidelines for Pain and Distress in Laboratory Animals***. 8**. Washington: OACU, (2010).
13. NEVES, S. M. P., PRATES, F. M.; RODRIGUES, L. D.; SANTOS, R. A.; FONTES, R. S.; SANTANA, R. O. *Manual de cuidados e procedimentos com animais de laboratório do Biotério de Produção e Expermentação da FCF-IQ/USP.* FCF-IQ/USP, São Paulo, Brazil (2013).
14. ROCHA, T. M. *Feridas e Estomas Em Oncologia – Uma Abordagem Interdisciplinar*. Fisiologia do Processo Cicatricial. Lemar, São Paulo, Brazil (2011).
15. SUNARTI, S. "Successful Treatment of UnstageablenPressure Ulcer by Using Advanced Wound Dressing". *Acta Med. Indones*. **Vol. 47**, n. 3, pp 251-2, (2015).