Effects of the application of *Aloe vera* (L.) and microcurrent on the healing of wounds surgically induced in Wistar rats[1]

Efeitos da aplicação de *Aloe vera* (L.) e microcorrente no reparo de lesões cirúrgicas induzidas em ratos Wistar

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ABSTRACT
Purpose: To investigate the effects of topical application of an *Aloe vera* gel combined or not with microcurrent application on the healing of skin wounds surgically induced in Wistar rats. **Methods**: The animals were randomly divided into the following groups: control group, animals topically treated with *Aloe vera*, animals treated with a microcurrent, and animals receiving topical application of *Aloe vera* combined with microcurrent application. **Results**: The results indicated differences in wound healing between the various treatments when compared to the control group. Tissue hyperplasia was lower in the control group compared to the other treated groups. Accelerated wound healing was observed in the group treated with *Aloe vera* compared to control. Animals submitted to microcurrent application only and the group treated with microcurrent plus *Aloe vera* presented an earlier onset of the proliferative phase compared to the control group and animals treated with *Aloe vera* gel alone. Morphometric data confirmed the structural findings. **Conclusion**: Simultaneous application of *Aloe vera* gel and microcurrent is an excellent choice for the treatment of open wounds thus indicating a synergistic action of these two applications
Key words: Wound Healing. Aloe. Electric Stimulation. Histology, Comparative. Rats.

RESUMO
Objetivo: Investigar os efeitos da aplicação tópica do gel de *Aloe vera*, combinada ou não com a aplicação de microcorrente no reparo de lesões cutâneas induzidas cirurgicamente em ratos Wistar. **Métodos**: Os animais foram distribuídos aleatoriamente em: grupo controle, tratado topicamente com gel in natura de *Aloe vera*, tratado com microcorrente e tratado com aplicação tópica de *Aloe vera* associada à microcorrente. **Resultados**: Os resultados do presente trabalho indicaram que o reparo tecidual ocorreu de forma diferenciada nos vários tratamentos empregados quando comparados ao grupo controle. A hiperplasia tecidual no grupo controle foi menor que a observada nos demais grupos tratados. No grupo tratado com aplicação de *Aloe vera* o processo de reparo foi acelerado em relação ao controle. Os animais do grupo tratado somente com microcorrente e do grupo tratado com microcorrente associada à *Aloe vera* apresentaram uma fase proliferativa mais precoce quando comparados com o grupo controle e tratado somente com *Aloe vera*. Os dados morfométricos confirmaram os achados estruturais. **Conclusão**: A aplicação simultânea do gel de *Aloe vera* e microcorrente é uma excelente escolha para o tratamento de feridas abertas indicando uma ação sinérgica dessas duas aplicações.
Descritores: Cicatrização de Feridas. Aloe. Estimulação Elétrica. Histologia Comparada. Ratos.

[1]Research performed at Laboratory of Micromorphology, Herminio Ometto University Center – UNIARARAS, Araras-SP, Brazil.

Introduction

The mechanism of tissue healing is a complex biological process that involves a perfect and coordinated cascade of cellular and molecular events promoting tissue reconstitution. This process arises as a response of the tissue to injuries induced by trauma or by surgical procedures. The process of wound healing is characterized by three phases that overlap and present a characteristic profile: inflammatory phase, proliferative phase and remodeling phase. Despite some recent advances in the understanding of these basic processes, wound healing disorders continue to cause diseases and even death. A wide variety of therapies has arisen with the

advances in technological applications. Electrical stimulation has been shown to modify the healing process in living organisms, especially in terms of factors that delay or impair this process[1,2].

Several studies have investigated cellular responses to electrical currents of different amplitudes and frequencies[2,3]. In this respect, Cheng and Goldman[2] and Kloth[4] observed that cells exposed to electrical fields vary in cell proliferation and metabolism. In *in vitro* studies, Goldman and Pollack[5] found that the application of electrical currents stimulated fibroblast proliferation and collagen synthesis. An increase of collagen biosynthesis, number of fibroblasts and hydroxyproline levels was also observed in animal experiments[6]. The technique of microamperage electrical stimulation is also called biostimulation or electrical therapy due to its capacity to physiologically stimulate cell growth.

The application of phytotherapeutic agents has also shown to be highly effective in the healing of wounds and burns[7]. *Aloe vera* (L.) (Liliaceae), popularly called aloe, is widely known for its therapeutic effects and has been used as a medicine since ancient times. "*Aloe vera*" was first called *Aloe perfoliata* var. *vera* by Linnaeus and was later recognized as a distinct species by Miller who called it *Aloe barbadensis*, and also by Burman who called it *Aloe vera*[8]. The plant originated in Africa and is well adapted to all regions. Two fractions can be extracted from its leaves: a bitter exudate and a mucilaginous gel. The first fraction is a yellow-red fluid extracted from cells of the pericycle which is rich in anthracene compounds. The second transparent gel-like portion (mucilage) originates from the leaf parenchyma and has been used for the treatment of burns and wounds because of its healing properties[9]. This mucilage consists of biologically active molecules that act on fibroblasts during the formation of cicatricial tissue, stimulating the deposition of collagen fibers in the extracellular matrix[10]. Reynolds and Dweck[11] investigated the biological activities of various *Aloe* species and observed that the whole gel extract of *Aloe vera* presents various pharmacological properties such as promoting wound, burn, and frost-bite healing, in addition to having anti-inflammatory, antifungal, hypoglycemic and gastroprotective properties.

In the present study, we investigated the effects of topical application of an *Aloe vera* gel combined or not with microcurrent application on the healing of skin wounds surgically induced in Wistar rats.

Methods

Experimental groups

Male rats from Wistar strain aged 120 days and with a mean weight of 250 to 350 g, obtained from the animal house of Hermínio Ometto University Center, Uniararas, were used. The animals were kept in individual cages, with commercial chow and water available *ad libitum*. In reason of similar genetic background of animals[12] and following orientation of Uniararas Ethics Commission (Protocol number 178/2006) were used four experimental groups with six animals each: control group, animals topically treated with *Aloe vera* gel (AV), animals treated with a microcurrent (10 μA/2 min) (MC), and animals receiving topical application of *Aloe vera* combined with a microcurrent (10 μA/2 min) (AV+MC). A Physiotonus Microcurrent apparatus was used for transcutaneous electrical stimulation.

Wound creation

The animals were submitted to trichotomy in the dorsal region and were anesthetized with 40 mg/kg body weight sodium pentobarbital. Next, a full-thickness skin surgical incision measuring 20 mm in length and 2 mm in depth was made in the skin of the back of the animal in the caudocranial direction. The incision was not sutured. The proposed treatments were started 24 h after the surgical intervention and were applied daily for 10 days. The animals were sacrificed on days 2, 6 and 10 for removal of the damaged area and structural and morphometric analysis. All surgical procedures and experimental design were performed by the same investigator in accordance with institutional ethical guidelines and following Rao and co-works[13] orientation.

Preparation and administration of Aloe vera *(L) N.L.Burm. (Liliaceae)*

The leaves of *Aloe vera* plants were collected daily in the morning in the Experimental garden of Hermínio Ometto University Center - Uniararas, Araras (SP), Brazil, and sent to the Laboratory of Physiology, Hermínio Ometto University Center, for use. For administration, an incision was made in the leaf and the mucilage was removed with a sterile swab and immediately applied to the skin wounds.

Collection and preparation of wound samples for structural analysis

Animals of the control and experimental groups were sacrificed with a sodium pentobarbital overdose on days 2, 6 and 10 for removal of the healing area for comparative structural and morphometric analysis. The complete wound region and the underlying muscle with 2 mm adjacent unwound skin were collected by excising a rectangular area measuring approximately 120-160 mm^2. Each sample was fixed in buffered formalin for 24h at room temperature. Next, the specimens were washed in buffer and processed for embedding in Paraplast™. Longitudinal sections (7 μm thick), obtained from the mid-region toward the margin, were stained with hematoxylin/eosin, picrosirius-hematoxylin and Toluidine blue, pH 4.0. The specimens were observed and documented under a Leica DM 2000 photomicroscope at the Laboratory of Micromorphology, Centro Universitario Herminio Ometto, UNIARARAS.

Histomorphometric analysis

The histomorphometry of skin wounds healing in control and experimental groups was analyzed by Sigma Scan Pro 6.0™ and Leica Image Measure™ softwares using digital images captured from slides stained by Toluidine blue. Images were captured, using digitalization system (total magnification 200X) from Leica DM 2000 photomicroscope. Three randomly chosen optical microscopic fields for each histological slide pair were stored and submitted to a count of total cells (fibroblastic and inflammatory cells) (x 10^3 μm^2), of newly formed blood vessels and measurement of wound healing area (x 10^3 μm^2) and thickness of the regenerating epithelium (μm).

2.6. Statistical analysis

Data obtained for the different experimental groups were stored in electronic spreadsheets and compared by the ANOVA and Tukey test (5% level of significance) using the Biostat for Windows XP™ program.

Results

In the present study we describe the repair of the epidermis and dermis, included morphometrical parameters, in wound healing between the various treatments when compared to control group.

Wound healing was completed in all animals within the 10-day observation period. Structural analysis was performed on days 2, 6 and 10. Tissue repair was observed 2 days after surgery (inflammatory phase) and was in the overt proliferative phase at 10 days in all experimental groups (Figures 1 and 2). Epithelization did not differ between the control and experimental groups. Discrete repair was observed two days after surgery in all groups. After 6 days, epithelial organization was similar to that observed at 10 days, but the wounds were not completely closed as observed for the last experimental period. With respect to the dermis, an accelerated wound healing was observed in the AV group (Figures 2A, B) compared to the control group (Figures 1A, B). The tissue of repair was lower in the control group than in the other treated groups. Animals of the MC group (Figures 1C, D) and AV+MC group (Figures 2C, D) presented an earlier onset of the proliferative phase than control animals and animals of the AV group. On the other hand, the area of newly formed tissue seemed to be lower in the MC group than in the AV+MC group.

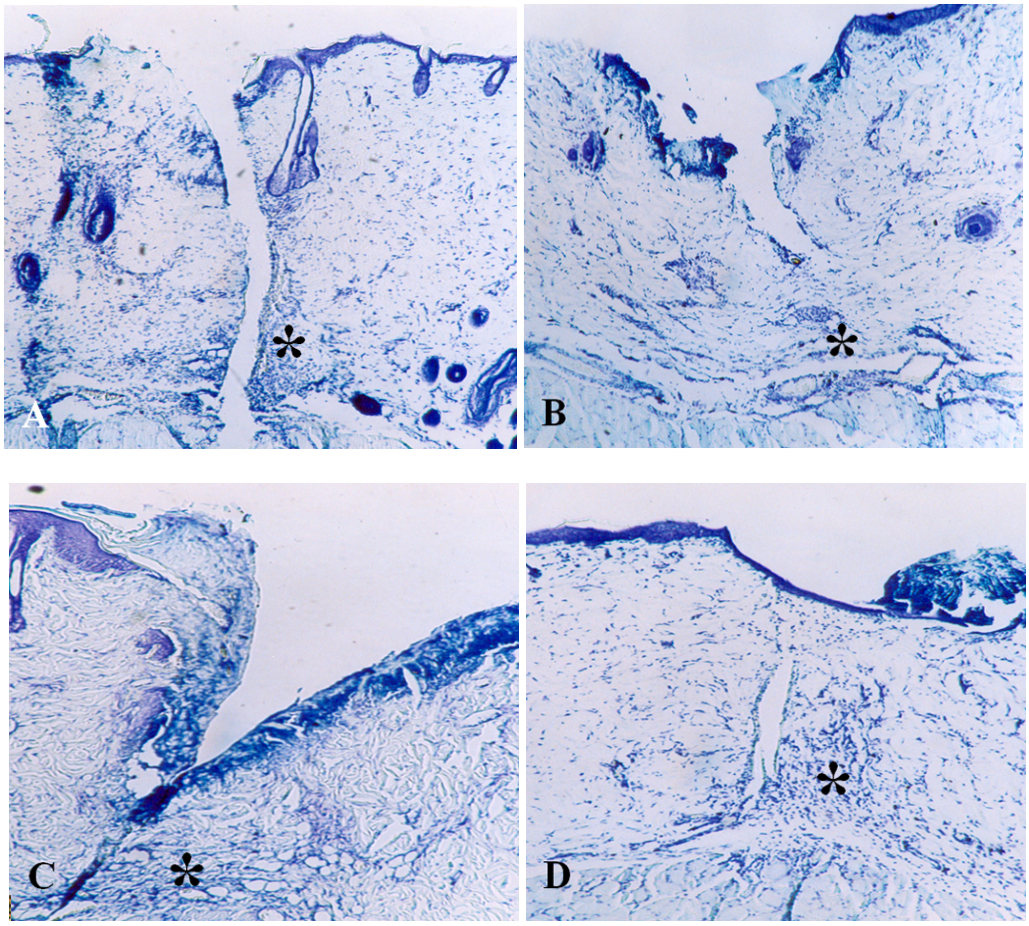

FIGURE 1 - Cross-sections of a surgical wound obtained from control animals at 2 (**A**) and 10 days (**B**) after the surgical procedure and from animals submitted to microcurrent application at 2 (**C**) and 10 days (**D**). The sections were stained with Toluidine blue. (*)Àrea of tissue healing. Final magnification: 200x

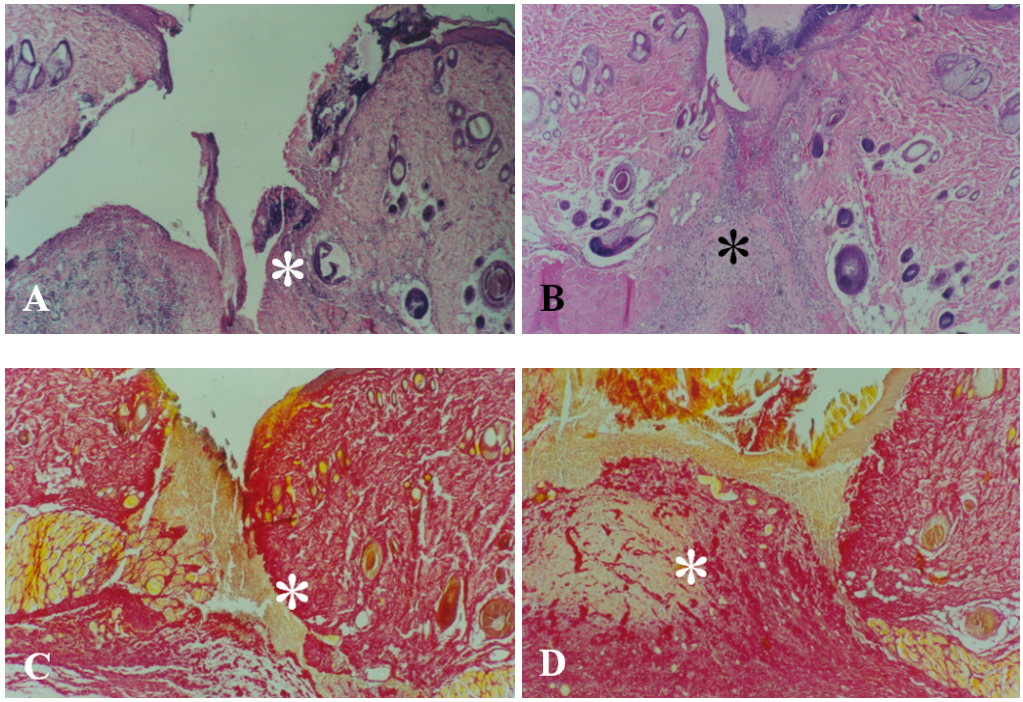

FIGURE 2 - Longitudinal sections of a surgical wound obtained from animals treated with *Aloe vera* at 2 (**A**) and 10 days (**B**) after the surgical procedure and from animals treated with *Aloe vera* and microcurrent at 2 (**C**) and 10 days (**D**). The sections were stained with hematoxylin/eosin (**A** and **B**) and picrosirius (**C** and **D**). (*)Àrea of tissue healing. Final magnification: 200x

The Figures 3, 4, 5 and 6 shows the morphometric data obtained for the different groups at 2, 6 and 10 days after experimental wound induction. On the second day after wound induction, a significant difference was only observed regarding the area of newly formed tissue, which was greater in the MC and AV+MC groups, with no significant difference between these two groups. The combined application of *Aloe vera* and microcurrent was found to be more effective in this process when compared to the control group and to the groups submitted to the application of either treatment alone. No blood vessel formation was observed during this period. After 6 days, the healing area was significantly greater in the MC and AV+MC groups, with no significant difference between these two groups. The same pattern was observed for the total number of cells and number of newly formed vessels. No significant difference in epithelial thickness was observed between groups. On day 10, the wound healing area was similar in the AV, MC and AV+MC groups and significantly greater than in the control group. On the other hand, the number of fibroblasts and the number of newly formed vessels were larger in the MC and AV+MC groups.

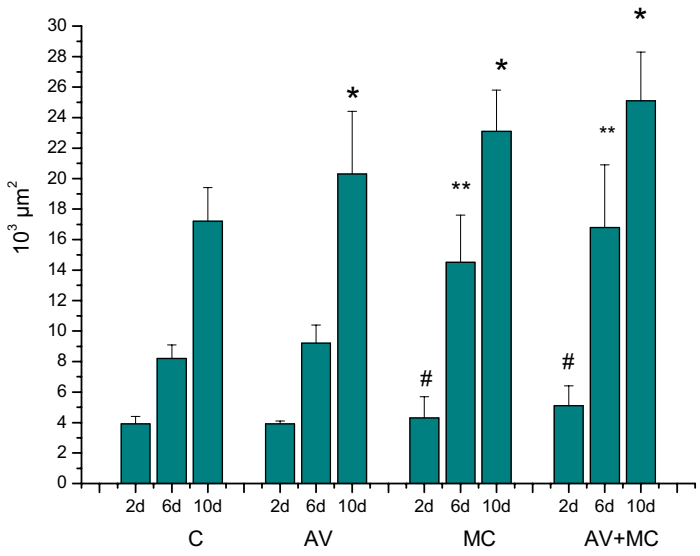

FIGURE 3 – Measurement of healing area in the region of the skin wound experimentally induced in normal 120-day-old Wistar rats after 2, 6 and 10 days of treatment. C: control; AV: animals topically treated with *Aloe vera*; MC: animals treated with microcurrent (10 µA/2 min); AV+MC: animals receiving topical application of *Aloe vera* plus microcurrent (10 µA/2 min). Values are reported as means ±SD. #, ** and * represent p<0,05.

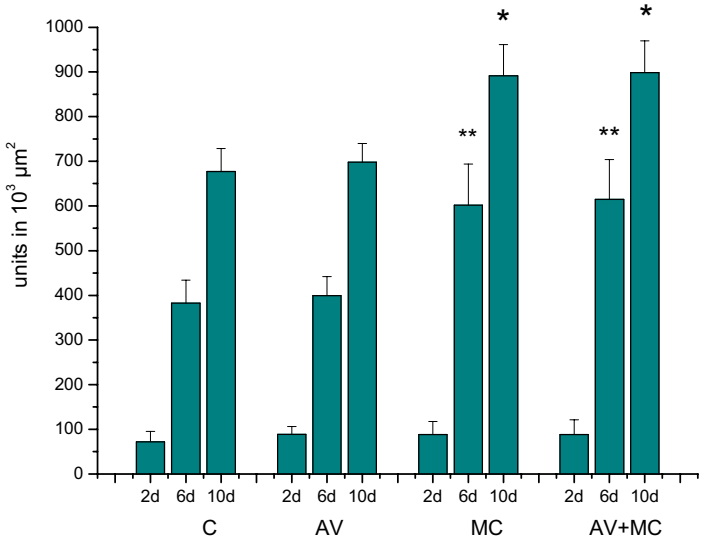

FIGURE 4 – Number of cells detected by 10^3 µm^2 of healing area in the region of the skin wound experimentally induced in normal 120-day-old Wistar rats after 2, 6 and 10 days of treatment. C: control; AV: animals topically treated with *Aloe vera*; MC: animals treated with microcurrent (10 µA/2 min); AV+MC: animals receiving topical application of *Aloe vera* plus microcurrent (10 µA/2 min). Values are reported as means ±SD. ** and * represent p<0,05

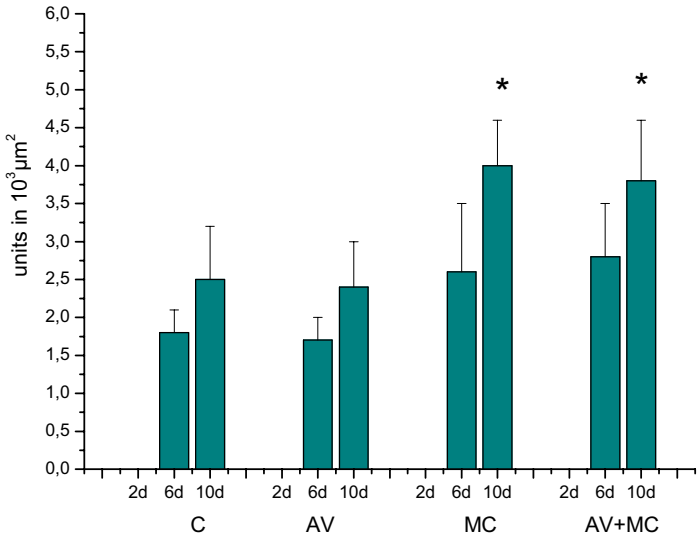

FIGURE 5 – Number of new vessels detected by 10^3 µm^2 of healing area in the region of the skin wound experimentally induced in normal 120-day-old Wistar rats after 2, 6 and 10 days of treatment. C: control; AV: animals topically treated with *Aloe vera*; MC: animals treated with microcurrent (10 µA/2 min); AV+MC: animals receiving topical application of *Aloe vera* plus microcurrent (10 µA/2 min). Values are reported as means ±SD. * represent p<0,05.

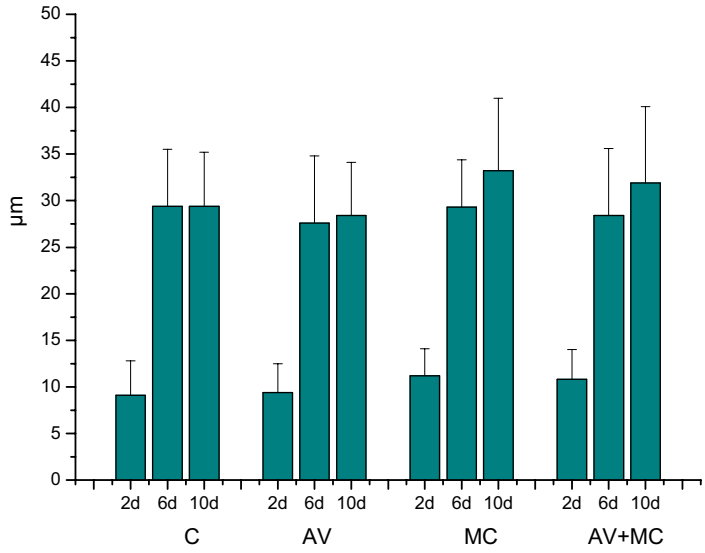

FIGURE 6 – Epithelial thickness measurement in the healing area of the skin wound experimentally induced in normal 120-day-old Wistar rats after 2, 6 and 10 days of treatment. C: control; AV: animals topically treated with *Aloe vera*; MC: animals treated with microcurrent (10 µA/2 min); AV+MC: animals receiving topical application of *Aloe vera* plus microcurrent (10 µA/2 min). Values are reported as means ±SD. No statistically significant results are detected

Discussion

In the present study, both microcurrent therapy and topical application of *Aloe vera* were effective in accelerating the tissue regeneration process. These findings agree with those reported in different studies demonstrating that low-intensity electrical currents stimulate wound healing[14,15]. Numerous investigators have studied the effects of electrical stimulation using different amplitudes and frequencies and have observed modifications in the cellular and tissue responses in induced lesions[4,16]. Some studies reported variations in cell metabolism, whereas others demonstrated fibroblast proliferation, neovascularization and collagen deposition in the wound area[17]. Significant acceleration of the healing process after microcurrent electrical stimulation has been widely documented[4,5,18,19]. Becker[14] reported that electrical currents are present in a biological system and may promote repair and growth after injury. According to this author, a specific stimulus of injury induces another stimulus of repair and also demonstrated that the membrane electrical potential is altered in injured tissues. The "injury signal" gradually decreases in parallel to the repair process and ceases when the latter is complete. The voltage peaks immediately after injury and gradually decreases as the wound heals, a fact leading to the concept that current flow may be defective in chronic wounds and that applying electrical currents to wounds may stimulate healing[4,20]. Biedeback[21] proposed that transmembrane currents open voltage-controlled calcium channels in fibroblasts, causing ATP resynthesis, activation of protein kinase mechanisms to synthesize new cellular protein, and DNA replication necessary for mitotic cell division.

In the present study, microcurrent application was found to be highly effective in terms of the parameters analyzed, with positive effects on the newly formed tissue area, number of fibroblasts, number of newly formed vessels and epithelial thickness, in agreement with the literature[4,21,22].

Similar, but less pronounced, effects were observed when *Aloe vera* extract was applied. Mello *et al.*[23] suggested that the healing capacity of *Stryphnodendron adstringens* might be attributed to the high tannin content in its stem bark. Tannins precipitate proteins in damaged tissues, forming a protective lining that favors healing by reducing wound permeability and exudation[24]. Sarabia *et al.*[25] demonstrated the presence of antioxidant substances in *Aloe vera* that confer anti-inflammatory and healing properties. Kuzuya *et al.*[26] also reported an antibacterial action of *Aloe vera* and attributed these properties to the presence of anthraquinones such as aloenin, barbaloin and iso-barbaloin in its chemical composition. Chitra *et al.*[10] also suggested that treatment with *Aloe vera* has a beneficial effect on the tissue proliferation phase, influencing fibroplasia and collagen synthesis and thus increasing the healing area. Mannose-6-phosphate is a major structural constituent of *Aloe vera*. Davis *et al.*[27] speculated that the binding of mannose-6-phosphate to fibroblast receptors activates fibroblast proliferation.

As observed in the present study, wound healing was more effective since the beginning of treatment in animals submitted to the simultaneous application of microcurrent and *Aloe vera*, thus indicating a synergistic action of these two applications. This treatment presented advantages in all parameters studied when compared to the control group and to the other treatments. These findings agree with Soares[28] who combined a chemical substance, i.e., vitamin C, and physical agents for the healing of experimental wounds similar to those used in the present study, and demonstrated that the combination of antioxidant agents and photodynamic or low-amperage electrical therapy accelerates wound healing.

Conclusion

Despite the lack of scientific studies regarding the combination of phytochemical and physical agents for the treatment of surgical wounds, the present investigation shows that the simultaneous application of *Aloe vera* and microcurrent was effective for the treatment of open wounds potentiating wound healing.

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