IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 23, Issue 10 Ser. 6 (October. 2024), PP 01-08
www.iosrjournals.org

Effect Of Low Frequency Microcurrents On Skin Wounds Of Rats"

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Abstract-
This study was conducted to verify the effect of low-intensity and low-frequency electric current using a bipolar symmetrical square waveform on cutaneous wounds in rats. The experiment used 3 groups of animals, all with a surgical wound measuring 8 mm in diameter on the back, being a control group, a second group treated with a current intensity of 30 µA (G30) and a third group treated with a current intensity of 160 µA (G160), for 30 minutes, immediately after the surgical injury. Groups G30 and G160 were stimulated with microcurrent using a frequency of 0.3 Hz and a pulse width of 1.6 s. All animals were clinically evaluated daily for 7 days after surgery, and then sacrificed. The injured region was biopsied, histologically processed and analyzed by a digital image processing system (Leica Qwin). Statistical analysis revealed that the G30 and G160 groups presented a significant reduction in inflammatory cells ($p<0.001$) in the superficial region of the dermis, when compared to the control group, and an acceleration in the proliferation of superficial fibroblasts ($p<0.05$) in relation to the control group. There was also a significant difference in the reduction in wound diameter, in the periods of 72 hours ($p<0.05$), 144 ($p<0.01$) and 168 hours ($p<0.05$) of the G160 group in relation to the control. The animals in the G30 group presented a significant reduction in wound diameter ($p<0.05$) in relation to the control group, after 144 hours of surgery.
Keywords: *Micro current, electrical stimulation, wound healing, physiotherapy.*

Date of Submission: 05-10-2024 Date of Acceptance: 15-10-2024

I. Introduction

Tissue repair is a phenomenon of great interest to health professionals. Its efficiency accelerates the rehabilitation process, with subsequent faster return to work, leisure activities and even social interaction (Ruiz-Silva et al., 2016). The change in the epidemiological profile and the increase in the population's life expectancy have led to a consequent increase in the complexity of surgical procedures. The prevalence of chronic diseases leads to the need for continuous treatments and the occurrence of dysfunctions and dependence, and accelerating the healing process reduces the length of stay in the hospital environment (Oliveira, 2019).

Normal tissue repair is an integration of dynamic interactive processes involving chemical mediators; blood formed elements; production of extracellular matrix and parenchymal cells. In the repair process, regeneration and healing should be considered as distinct events, however, both processes are linked, occurring together. Healing events can be divided into inflammation, the phase of formation of granulation tissue with deposition of extracellular matrix and tissue remodeling (Ruiz-Silva et al., 2016; 2023).

Most textbooks on wound healing and tissue repair fail to discuss either the physiology of natural endogenous electrical currents in wound healing or the application of external (exogenous) electrical currents to accelerate healing and tissue repair. (Ruiz-Silva et al., 2006, Couto; Ruiz-Silva, 2010; Lee, 2024)

When there is a loss of continuity of the superficial tissue, an electrical alteration occurs, called the injury current by Matteucci (1830), Dubois-Reymond (1843) and Barker (1980), and the endogenous electricity bypasses the area of injury, for Nordenstron healing comes from a small electrical stimulus applied to the injured area (Ruiz-Silva et al., 2016). Studies report the effect of low-intensity electric current on wound healing, which resulted in the acceleration of the healing process, and suggest an increase in the healing speed (Avendanõ-Coy, 2022), but the effects, parameters and standardization of currents have not yet been established, with controversial data regarding the intensity and waveform of the current. The use of microcurrent therapy on skin lesions aims to normalize the flow of currents, aiming at repair and minimizing pain; evidence confirms the migration of epidermal cells, fibroblasts, leukocytes and macrophages by electric fields (Ruiz-Silva et al., 2016; 2023). This work is a collaboration in the search for specifying the most effective parameters in the application of microcurrent as an adjuvant factor for reducing wound healing time and attenuating the symptoms caused by these lesions, aiming at better quality of the scar tissue.

II. Materials And Methods

This study used 21 male Wistar rats (Rattus norvegicus variety albinus), which were weighed on a Bender® analog scale, verifying that their body weight was between 200 and 250 g. The animals remained for a 15-day acclimatization period in the animal facility of the Research and Development Institute (IP&D) of the University of Vale do Paraíba. They were kept in cages (five animals per cage) under environmental conditions of temperature and light, fed a standard Labina® diet and water ad libitum. The experiment was carried out in the Tissue Biomodulation and Fluorescence Laboratory of the Research and Development Institute (IP&D) of the University of Vale do Paraíba. The animals were divided into a control group (CG) (n=9) and two groups treated with microcurrent therapy, one treated with a current intensity of 30 µA (G30) (n=6) and the other with a current intensity of 160 µA (G160) (n=6). The animals were weighed and administered the pre-anesthetic Butorphanol (Torbugesic®, 2mg/kg) associated with Acepromazine 0.2% (Acepran®, 1mg/kg), both administered in a single dose, intramuscularly. After 15 minutes, Zolazepan and Tiletamine (Zoletil 50®, 40 mg/kg) were administered. The animals were trichotomized on the right dorsal region. A circular incision was made with the aid of a sterile "punch" biopsy instrument, with a diameter of 8 mm, in the trichotomized region (Figure 1).

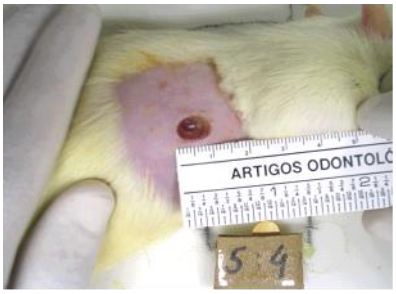

Figure 1: Skin lesion on the back of a rat (0.5 cm2).

To carry out this study, a micro electrostimulation device was used, a prototype that gave rise to Phasys KLD biosystems, the MTC, with a constant current output amplifier, with two channels, square waveform, biphasic, symmetrical balanced. The animals in groups G30 and G160 received therapy, with current intensity of 30 µA and 160 µA, respectively, for 30 minutes immediately after the surgical injury, with electrodes applied in the quadripolar form, with a frequency of 0.3 Hz with a pulse width of 1.6 s (Figure 2).

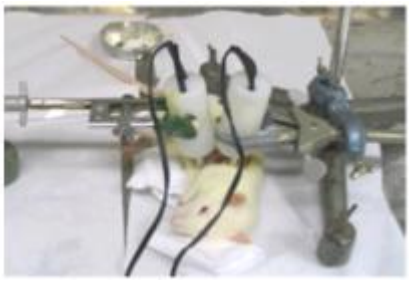

Figure 2: Animals receiving Microcurrent Therapy.

The animals in the control group did not undergo any type of post-surgical therapy and were maintained as controls. The injured region of the animals was monitored daily through clinical evaluation (wound diameter and presence of edema, exudate and/or crust). Seven days after injury, all animals were anesthetized and sacrificed with a lethal dose of 10% potassium chloride (KCl, 4 ml/kg of body weight). The wound diameter values were expressed as mean and standard error, and submitted to the ANOVA statistical test. The significance level established was 5% ($p<0.05$). To qualify and quantify the representative areas of inflammatory cells, fibroblasts, neovascularization and collagen, three fields per slide were digitized, for a total of three slides per animal. All digitized images were standardized according to the light intensity of the microscope and the height of the condenser. To define the measurement quadrant, the Frames were standardized in: line H = 280 and line W = 280, in order to obtain a quantified image in 50,805 µm2, calibrated in Ipixel = 0.805 µm, then the area occupied by inflammatory cells, fibroblasts, adipocytes when present was calculated with the help of the Microscope and Leica Qwin program. The injured tissue was analyzed in two distinct portions, divided into two subsequent quadrants of 50,805 µm2, one starting from the surface of the dermis is called superficial and the other, just below it, is called deep dermis.

To analyze the statistical results, the Graf Pad Instat® program was used applying the Bonferroni multiple comparison test, and we obtained the results that are expressed in the graphs below.

III. Results

The results of the analysis of wound diameters 7 days after surgery are summarized in Figure 3.

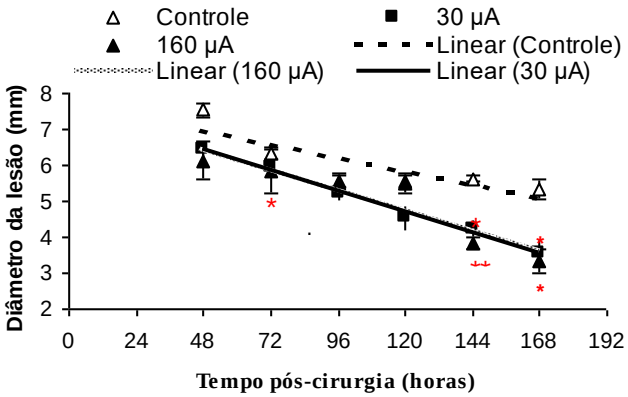

Figure 3: Lesion diameter in the different groups between 48 and 168 hours post-surgery. Data are expressed as mean ± error (* p<0.05, **p<0.01 compared to control).

In the 48-hour period, there was a reduction in the diameter of the wound in the animals stimulated with TMC (G30=6.42 mm2 and G160=6.13 mm2). It can be noted that the animals presented a rapid contraction of the wound in relation to the control (GC=7.54 mm2). In 96 hours, all groups presented a similar average diameter (GC=5.54 mm2, C30=5.25 mm2 and C160=5.50 mm2). After the detachment of the crust after 120 hours, the group treated with 30 □A evolved better than the others (GC=5.55 mm2, C30=4.53 mm2 and C160=5.50 mm2). At the end of the experiment, the stimulated groups presented a superior closure than the control (GC=5.35 mm2, C30=3.54 mm2 and C160=3.33 mm2). The 160 □A group, compared to the Control group, showed significant differences in the reduction of the lesion diameter in the periods of 72 hours ($p<0.05$), 144 ($p<0.01$) and 168 hours ($p<0.05$), while the 30 □A group showed significant differences in the periods of 144 and 168 hours ($p<0.05$).

In the histological analysis, it was observed that in the superficial portion of the control group there was a predominance of inflammatory cell population, with an average count of 2,887 cells corresponding to 5.7% of the measured area, in relation to 779 cells and 1.6% of the area in G30 and 148 cells and 0.3% of the area in group G160, where through the ANOVA test a statistically significant difference was found between the control and the treated groups ($p<0.001$), figure 4.

In the region of the dermis called deep (second quadrant of 50,805 µm2), inflammatory cells were observed in all groups, however without statistical difference between them.

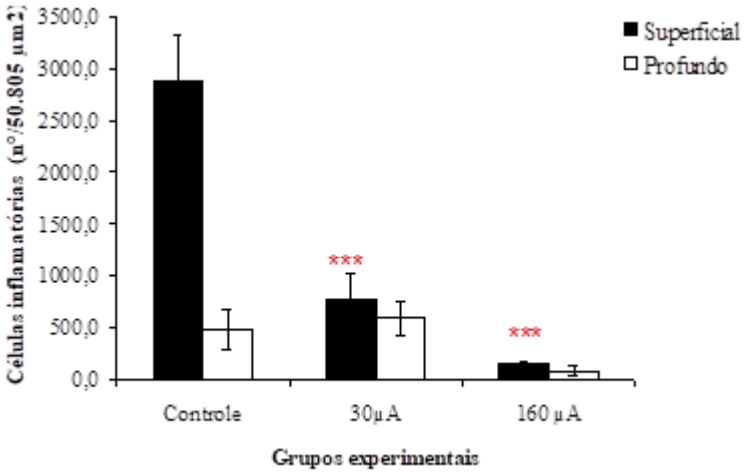

Figure 4: Number of inflammatory cells in the superficial and deep dermis (per area of 50,805 µm2) of the treated and control groups. Data are expressed as mean ± error (* p<0.001 in relation to the control group).**

The results of the fibroblast count were graphically described in Figure 5.

A significant difference ($p<0.05$) was observed in the number of superficial fibroblasts in the dermis in group G160 (2,295 cells/50,805 µm2) compared to group GC (504 cells/50,805 µm2), while group G30 (1,276 cells/50,805 µm2) did not present significant differences.

In the subsequent deeper region, an increase in the number of fibroblasts was found in all groups (G160 = 2,034 cells/50,805 µm2, G30 = 990 cells/50,805 µm2, GC = 1,585 cells/50,805 µm2), with no significant differences. In the fibroblast count, the results described graphically in figure 5 were found.

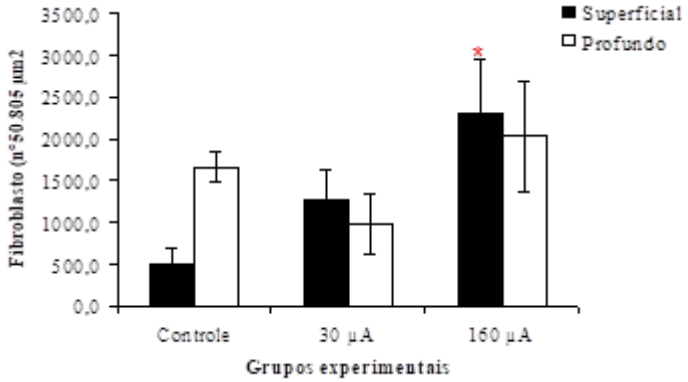

Figure 5: Number of fibroblasts found in the superficial and deep dermis (per area of 50,805 µm2) of the treated and control groups. Data are expressed as mean ± error (* $p<0.05$ in relation to the control).

Figure 6 shows the counts of the number of blood vessels found in each region evaluated in the groups of this experiment.

Angiogenesis was observed in all groups, being more pronounced superficially in the treated groups, in the deep region in the control group, but without significant results.

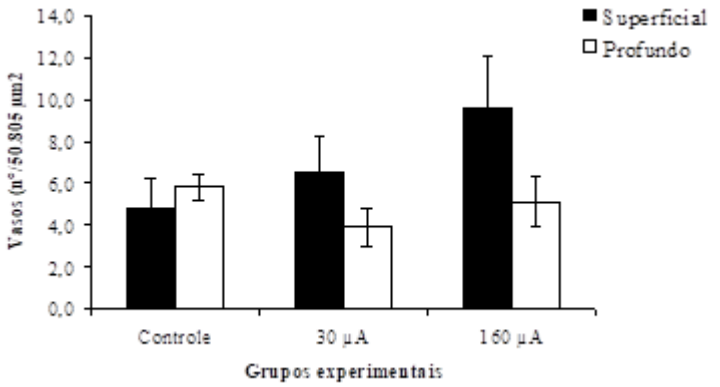

Figure 6: Number of vessels found in the superficial and deep dermis (per area of 50,805 µm2), of the treated and control groups.

In the analysis of the degree of evolution in the qualitative healing of the samples, we used aspects proposed by Wei Yu (1997).

In the untreated group (CG), minimal re-epithelialization was observed, without collagen deposition (Figures 7), with granulation tissue with a marked predominance of superficial lymphocytic inflammatory infiltrate, demonstrating a significant difference in relation to the treated groups ($p<0.001$) (Figure 7), discrete neovascularization in the deep dermis, and a discrete fibroblastic proliferation, containing cells with oval nuclei in the deep portion of the dermis (Figures 8). Atypical adipocytes were also observed on the surface in three animals (33.33%).

The degree of evolution in healing, attributed in the analysis of the animals in the CG group, obtained an average of 4.4 ± 0.4%.

Figure 7: Photomicrograph of the control group. Presence of predominantly superficial inflammatory cells (arrows). H&E, 100x.

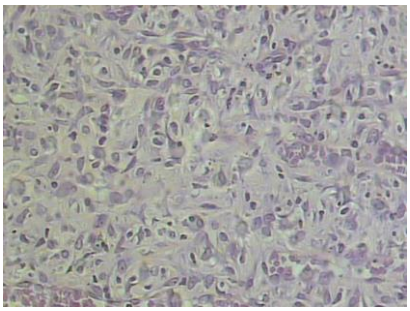

Figure 8: Photomicrograph of the control group. Fibroblasts with oval nuclei can be observed (arrows). H&E

In the dermis of specimens from group G30, the presence of granulation tissue was observed, less frequent than in the deep region of the dermis, and intercellular matrix in greater quantity than in group GC. A discrete proliferation of dilated and congested capillaries was also observed on the surface and lower quadrant of the dermis (figure 9), demonstrating neovascularization and a discrete presence of inflammatory infiltrate in the deepest portion of the dermis (figure 9).

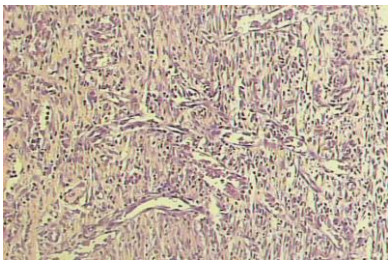

Figure 9: Photomicrograph of the group stimulated with 30 µA. Dilated and congested vessels (arrows) and some inflammatory cells can be observed.

A common aspect to all animals was the proliferation of fibroblasts characterized by mature cells whose nuclei were flattened (figures 10) and the deposition of collagen in the center of the lesion, more pronounced in the reticular region (figure 11) in the lower quadrant of the dermis. H&E, 200x.

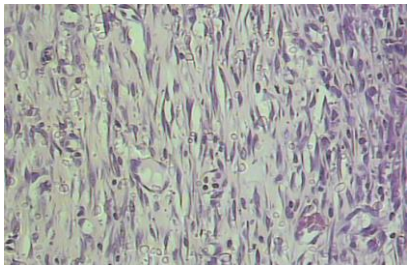

Figure 10: Photomicrograph of group G30. Granulation tissue and fibroblasts characterized by spindle cells composed of flattened nuclei (arrow) can be observed, H&E, 400x

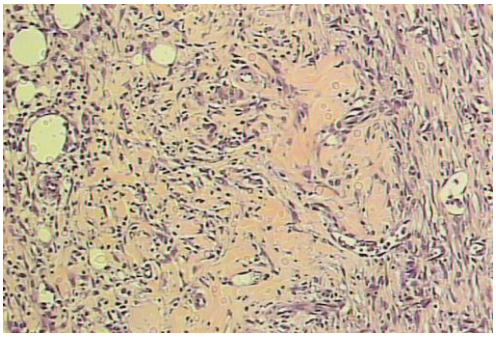

Figure 11: Photomicrograph of group G30. Collagen deposition can be seen in the center of the lesion, more pronounced in the reticular region (arrows), H&E, 200x.

The degree of healing evolution, attributed in the analysis of group G30, obtained an average of 8.7 ± 1.3%.

In the analysis of group G160, intense neovascularization was noted, with capillaries located close to the surface, where the presence of dilated and congested capillaries was observed, but no statistical differences were observed.

The inflammatory infiltrate observed was discreet. The granulation tissue was characterized sometimes by intense proliferation of mature fibroblasts (Figure 12) distributed in a dense connective tissue richer in dense collagen fibers with orientation parallel to the surface of the repair area, and sometimes by the presence of greater vascularization, but with less intensity of hyperemia than that observed in the other groups, and in a much greater quantity than the group stimulated with 30 µA. They presented a large quantity of dense collagen composed of connective tissue in the process of maturation, with superficially disorganized fibers and already aligned in the deepest portion (Figure 13). Regarding the epidermis, a paving of epithelial cells covering the wound was observed in all animals in this group, characterizing thick epithelialization.

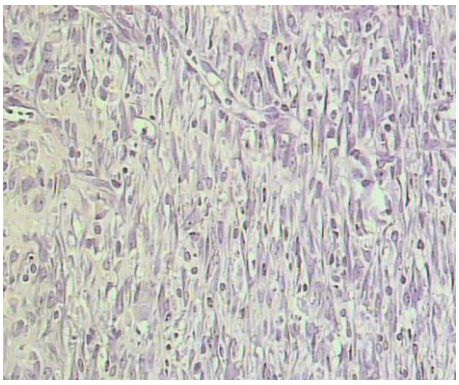

Figure 13 - Photomicrograph of the group stimulated with 160 µA. Dense collagen composed of connective tissue in the maturation process can be observed, with superficially disorganized fibers and aligned in the deepest portion (arrows), H&E, 200x.

This group presented statistically significant results regarding the number of superficial fibroblasts ($p<0.05$) and a decrease in inflammatory cells ($p<0.001$) when compared to the control group.

The degree of healing evolution, attributed in the analysis of the animals in this group, obtained an average of 12.7 ± 0.3%, according to .

IV. Discussion

Tissue repair is one of the most important and interesting phenomena in living beings. Repair, in its most comprehensive form, represents the organism's effort to maintain a stable state. Ideally, repair should lead to the formation of morphological structures that can continue to perform physiological activities and functions.

Several studies report the effects of the use of low-intensity electrical current in the healing of incisional and excisional wounds. In the 1960s, Assimacopoulos (1964) obtained dense connective tissue rich in hyalinized collagen fibers arranged in parallel, using microcurrent treatment. In the present study, we found the same results with just one post-surgery application. Based on the results found by Assimacopoulus (1964; 1968), several studies, both in vivo (Ruiz-Silva, 2006; 2016) and ex vivo (MITCHELL, 1976; CHENG, et al. 1982), were carried out to demonstrate the efficiency of the technique and mechanisms of physiological action, supporting the working hypothesis that TMC can accelerate the healing process. Several authors suggest an increase in the speed

of contraction, closure and healing of the wound in the first few days with the use of microcurrent (Ruiz-Silva, 2006; 2016), a result obtained in our clinical analysis in the treated animals compared to the control. Despite previous studies, the effects, parameters, and standardization of currents have not yet been established. The literature analyzed shows controversial data regarding the intensity and waveform of the current (Ruiz-Silva, 2006; 2016), but all cases showed positive results in the synthesis of collagen in the dermis, epidermis, and in the thickness of the wound, using currents with intensities between 10 and 300 µA, which is why two intensities in this range were established in this experiment, that is, 30 µA and 160 µA (Xu, 2021).

The results of the present study suggest that the control animals presented pathophysiological characteristics consistent with normality, which would result in conventional healing by secondary intention. On the other hand, even though the general picture observed in the treated group also showed a tendency towards complete healing of the lesions, this was different when comparing the results to the control group or to each other (30 µA and 160 µA), showing that the use of TMC resulted in biostimulation of wound healing, corroborating citations by Becker (1995).

A significant decrease in inflammatory signs was observed in the treated animals in relation to the control group. These aspects may represent an anti-inflammatory effect of TMC, since there was wound retraction, early development of the scab, reduction of edema, and no animal presented exudate and pus when stimulated, corroborating Morgareidge and Chipman (1990) who state that by increasing the electric field of the wounds we obtain the migration of epidermal cells, fibroblasts, leukocytes and macrophages to the wounded margin.

Monetta (1998) reports that the scab interferes with the contraction of the wound, hindering the healing phase. In this study, the treated animals developed a crust in the first few hours, and all animals had a crust within 96 hours, with the animals in the 30 µA group losing it quickly.

Another important fact to be considered was the presence of intense fibroblast proliferation in the treated groups, especially those stimulated with 160 µA, when compared to the control, a fact that was demonstrated by Feng et al. (1983), where they indicated greater fibroblast activity. It was demonstrated that fibroblasts are aerobic cells and require oxygen both for division and for collagen synthesis.

We can observe a greater number of spindle-shaped fibroblast cells, cells that characterize a mature tissue in the group treated with 160 µA in relation to the group stimulated with 30 µA. This aspect indicates an acceleration in the fibroblast proliferation process of the specimens treated with 160 µA. Fibroblast proliferation was lower in animals in the control group whose cells had oval nuclei, which characterizes a younger tissue, represented by slower healing when compared to the treated group (ABLA, 1995). It was observed that the connective tissue was richer in collagen fibers when stimulated at low intensity, more evident at 160 µA, which would again be explained by the results observed by Cheng et al. (1982) and confirmed in previous studies (Ruiz-Silva, 2006; 2016)

The group treated with 160 µA showed more intense neovascularization than the other groups, although the results were not statistically significant, and this was associated with intense fibroblast proliferation, where the greater the vascularization, the greater the blood supply. This aspect may represent the manifestation of the effect of TMC on the endothelial tissue, which could be the result of a greater release of chemical mediators of cell proliferation as already reported in previous studies (CHENG et al., 1982).

The inflammatory process at the end of seven days was less intense and apparently more evolved in the treated wounds than in the control ones, and even lower when treated with 160 µA. This could be explained by the earlier onset of the process in stimulated wounds. Young (1988) states that during the proliferative phase the number of inflammatory cells decreases, while the number of endothelial cells and fibroblasts increases. The latter are of great importance in skin repair, not only because they are the main producers of the extracellular matrix, but also because they contract, reducing the size of the lesion (YOUNG, 1988).

A deficient inflammatory response, as well as the decrease in fibroblasts in the wound, greatly reduces collagen deposition (SIMÔES et al., 1985). The results observed in animals treated with TMC demonstrate the efficiency of the technique in reducing the inflammatory response and stimulating collagen synthesis.

According to the statistical results, the groups treated with microcurrent, 160 µA and 30 µA, presented significant differences ($P<0.001$) in terms of the reduction of superficial inflammatory cells, demonstrating the acceleration of the superficial healing process in the groups treated with TMC, and that they are in a more advanced stage of healing, especially the group stimulated with 160 µA. This result is justified by the advanced stage of repair of these injuries, as the literature shows that cell proliferation decreases with a gradual reduction in the size of fibroblasts. At the same time, there is a slow increase in the elastic resistance of the wound, as the collagen fibers undergo greater interconnection, increasing their thickness and compaction (Ruiz-Silva, 2006; 2016).

According to the qualitative analysis (WEI YU, 1997), a gain in the healing process was obtained with the use of microcurrents; the group treated with 160 µA obtained a 65.4% gain and the group treated with 30 µA 49.5% in relation to the control group. The results corroborate studies carried out in humans, where the treated

group showed an improvement in the healing rate of 1.5 to 3.5 times in the speed of the healing response than the control group (WATSON; 1998).

The results obtained allow us to state that microcurrent should soon become a continuous use modality for healthcare professionals. However, knowledge of dosimetry and mechanisms of action of this technique require more detailed research studies.

V. Conclusions

The use of CMT with intensities of 30 □A and 160 □A, and frequencies of 0.3 Hz and 0.8 Hz, promotes, in a single session immediately after surgery, acceleration of the healing process, when analyzed clinically.

The histological analysis showed statistically significant results regarding the effectiveness in reducing the number of inflammatory cells in both treated groups (30 □A and 160 □A), and also in increasing the number of superficial fibroblasts with the use of 160 □A current.

We can state that it is an excellent option for the immediate post-surgical period, but we must conduct new studies with a larger number of animals and a longer evaluation period, as well as a study applying micro cellular therapy every 48 hours, in order to use the therapy as a treatment protocol.

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