The effect of microneedling on scars resulting from induced cutaneous injuries in rats

Efeito do microagulhamento na cicatriz de ferida cutânea induzida em ratos

ABSTRACT

Introduction: Being healthy corresponds to a state of absolute well-being. Scars are examples of conditions that endanger patients’ emotional integrity, compromising their quality of life. Consequently, there are several therapeutic alternatives aimed at alleviating aesthetic disorders. Microneedling stimulates collagen production, improving the healing effect in the induced cutaneous injury.

Objective: to evaluate the effect of microneedling in the scars of surgically induced cutaneous wounds in rats.

Methods: Twenty-four male rats were distributed in five study groups. A surgically induced incision was inflicted on the animals’ dorsa in all study groups, with the healing processes being followed up until completion. The study groups GC-14 and GC-30 served as controls for the groups GCM-14 and GCM-30, respectively.

Results: Reepithelialization and absence of granulation tissue were identified in 100% of the groups. Regarding the proportion of fibrosis, mean reductions of 19% and 4% were observed in GCM-14 and GCM-30, respectively. There was a stimulus to the production of type I and III collagen in the groups that underwent microneedling, with a greater amount of type I collagen in GCM-14 (62.1%) as compared to its control (37.8%).

Conclusions: Microneedling was effective in stimulating increased production of collagen fibers in 14 days, suggesting this treatment tends improve scars.

Keywords: Cicatrix; Collagen; Models, animal

RESUMO

Introdução: A saúde representa estado de completo bem-estar. As cicatrizes são exemplos de afeções que colocam em risco a integridade emocional do paciente, comprometendo sua qualidade de vida. Existem diversas alternativas terapêuticas visando amenizar distúrbios estéticos. O microagulhamento estimula a produção de colágeno, melhorando o efeito cicatricial da lesão provocada.

Objetivo: avaliar o efeito do microagulhamento na cicatriz de ferida cutânea induzida cirurgicamente em ratos.

Métodos: Foram utilizados 24 ratos, machos, distribuídos em cinco grupos de estudo. Em todos os grupos realizou-se a incisão cutânea induzida cirurgicamente no dorso do animal, aguardando-se a cicatrização completa. Os grupos GC-14 e GC-30 foram controles para os grupos GCM-14 e GCM-30.

Resultados: Foi identificada reepitelização e ausência de tecido de granulação em 100% dos grupos. Com relação à proporção de fibrose, observou-se redução média de 19% no grupo GCM-14 e de 4% no grupo GCM-30. Houve estímulo à produção de colágeno tipo I e III nos grupos submetidos ao microagulhamento, observando maior quantificação de colágeno tipo I no grupo GCM-14 (62,1%) em relação a seu controle (37,8%).

Conclusões: Microneedling foi eficaz ao estimular maior produção de fibras colágenas em 14 dias, sugerindo tendência à melhora da cicatriz.

Palavras-Chave: Cicatriz; Colágeno; Modelos animais
INTRODUCTION

There are currently several therapeutic alternatives intended to repair damages resulting from elective surgical procedures or trauma, which have left aesthetic disorders, such as normotrophic scars. Consequently, there is a trend towards the indication of non-invasive procedures, isolated or in association, aimed at alleviating these disorders, with a view to reducing the risk of complications and allowing earlier return to work activities.\(^1\)

Orentreich et al. (1995) \(^2\) described the term “subcision” as a means of stimulating connective tissue underneath scars and retracted wrinkles. Based on this idea, collagen induction therapy (CIT) or microneedling was developed as a technique performed using a device containing a variable number of microneedles, with different lengths, that cause cutaneous microtrauma with multiple perforations in the skin, resulting in the formation of microchannels. This device is rolled on the skin in multiple cross directions several times, causing minimal bleeding, which is replaced by serous exudate, edema and erythema.\(^3\)

The microneedling principle proposes the stimulation of collagen production, without causing total de-epithelization, in a fast, minimally invasive and effective manner, with promising results of the technique having been observed in a number of studies.\(^4-6\) This procedure generates a healing wound response by releasing growth factors and cytokines, which in turn lead to the formation of new collagen and elastin in the papillary dermis.\(^7\) The mechanism of action centers on the dissociation of keratinocytes, which results in the release of cytokines (mainly interleukin-1\(\alpha\), in addition to interleukin-8, interleukin-6, TNF-\(\alpha\) and GM-CSF - granulocyte and macrophage colony stimulating factor), resulting in dermal vasodilation and migration of keratinocytes in order to restore epidermal damage. The healing process resulting from the trauma caused by the needles can be divided into 3 phases.

In the first phase (injury phase) - presence of platelets and neutrophils (responsible for the release of growth factors that act on keratinocytes and fibroblasts), transforming growth factors \(\alpha\) and \(\beta\) (TGF-\(\alpha\) and TGF-\(\beta\)), platelet derived growth factor (PDGF), connective tissue activator protein III, and connective tissue growth factor.\(^8\)

In the second phase (healing phase), neutrophils are replaced by monocytes, and angiogenesis, epithelization and proliferation of fibroblasts take place, followed by the production of collagen type III, elastin, glycosaminoglycans and proteoglycans. Concomitantly, fibroblast growth factor, TGF-\(\alpha\) and TGF-\(\beta\) are secreted by monocytes. Approximately five days after the injury has been inflicted, the fibronectin's matrix is formed, allowing the deposition of collagen just beneath the basal layer of the epidermis.

In the third phase (maturation phase), type III collagen, which was predominant in the early stage of the healing process, is slowly replaced by type I collagen, which is more durable, and lasts for a period ranging from 5 to 7 years.\(^5\)

Therefore, the microneedling technique would act to stimulate the production of collagen, consequently improving the cicatricial effect of the lesion inflicted. This technique would be an alternative that would enable and potentialize the treatment of normotrophic scars.

OBJECTIVE

To evaluate the effect of microneedling on surgically induced cutaneous wounds in Wistar lineage rats.

METHODS

Sample

In the present experimental study, 24 male Rattus norvegicus (Wistar), aged 100 days, weighing on average 250 to 300g, were made available by the Instituto Evandro Chagas’ bioterium after the approval by the Ethics Committee on Animal Use – CEUA of the Universidade do Estado do Pará, Belém do Pará (UEPA), Pará, Brazil. Rats bearing active infectious processes or wounds that could compromise the cutaneous site where experiments would be carried out were excluded from the study. The animals were kept in a temperature-controlled environment before and after the procedure, with 12 hours of light cycle, water and rat-specific feed, offered ad libitum.

The animals were randomly assigned to five study groups. In all groups, the skin incision was surgically induced on the animals’ dorsa (described in the surgical technique section). Subsequently, a period of 3-week waiting period was observed in all groups for the complete healing of the surgical wound by second intention. From that moment on, differentiation in the procedures took effect according to the protocol established for each experimental group.

Group 1 – Pilot Group (Gp)

Four animals were used in this group after having undergone the pre-operative and anesthesia procedures, surgical technique and postoperative procedures specific to each of the four groups described below. They were not included in the research’s casuistry, having been used to improve the study researchers’ surgical technique.

Group 2 – Group Scar 14 days (GS-14)

Five animals were used in this group. Once the incision was performed and the complete healing period of the surgical wound elapsed (3 weeks), the animals underwent euthanasia 14 days after complete healing. The microneedling technique was not performed in this group, since it served as a control for comparisons with the other groups.

Group 3 – Group Scar and Microneedling 14 days (GSM-14).

Five animals were used in this group. Once the incision was performed and the complete healing period of the surgical wound elapsed (3 weeks), the animals underwent microneedling technique in the healing wound’s site, and euthanasia 14 days after the procedure.
Group 4 – Group Scar 30 days (GS-30)

Five animals were used in this group. After the incision was performed and the complete healing period of the surgical wound elapsed (3 weeks), the animals underwent euthanasia 30 days after complete healing. The microneedling technique was not performed on this group, which served as a control for comparison with the other groups.

Group 5 – Group Scar and Microneedling 30 days (GSM-30)

Five animals were used in this group. Once the incision was performed and the complete healing period of the surgical wound elapsed (3 weeks), the animals underwent microneedling technique in the healing wound’s site, and euthanasia 30 days after the procedure.

PROCEDURES

Preoperative and anesthesia

The animals were anesthetized with ketamine (70mg / kg) and xylazine (10mg / kg), by intraperitoneal route, with confirmation of anesthesia based on the absence of the podalic reflex, through interdigital pressure, as well as absence of the paw pinch reflex.

Microneedling

Dr. Roller® devices with 2.5mm long micro-needles, which are recommended when deep skin injury is required therefore being used to treat depressed scars, were used for the microneedling technique in GSM-14 and GSM-30 groups. In order to perform the technique, the device was positioned between the index finger and thumb, with moderate force being applied while moving it in four directions (horizontal, vertical and diagonals, ten passes in each), up until a uniform petechiae pattern was obtained. Aiming at minimizing a possible bias, the procedure was performed on all animals by the same person. When necessary, hemostasis was performed by tamponage with sterile gauzes.

Surgical technique

The animals were anesthetized and fixed with a surgical tape (20x30cm) in the ventral decubitus position. Trichotomy of the dorsal region and subsequent antisepsis of the surgical area were performed with 2% chlorhexidine.

A cutaneous incision of 4cm in length was surgically induced in all animals using a cold scalpel on the right dorsa. The incision extended from the most superficial layer of the skin to the limit of the subcutaneous cellular tissue, having as anatomical references the distance of 2cm from the spine and 4cm from the tail of the animal.

After this surgical incision, 3 weeks were awaited up until second intention healing was complete. This period was based on the observation of the animals in the Pilot Group, which suggested this was the period necessary for epithelialization and contraction of the wound. No suture or other synthesis technique was performed allowing the formation of a visible scar that favor the visualizing the microneedling’s effect. From this moment on, there were variations of the procedures according to the protocol established for each experimental group.

All groups (GS-14, GSM-14, GS-30 and GSM-30) underwent similar cutaneous incisions with second intention healing, as described above. After 3 weeks, all animals in GSM-14 and GSM-30 underwent microneedling. The euthanasia of the animals in GSM-14 was performed 14 days later, while in GSM-30 it was carried out 30 days after the microneedling procedure.

The animals in GS-14 and GS-30 did not undergo microneedling, with euthanasia being performed after 14 days in the first group and after 30 days in the second group, counting from the day it was performed in the other groups. In this manner, these groups were used as controls for comparison with the groups that underwent the microneedling technique.

The present study was aimed at analyzing and comparing the amount of collagen present in the cutaneous scar 14 days and 30 days after microneedling, since according to the literature, there is an increase in the levels of cytokines that induce formation of collagen in the period of 2 weeks after the procedure. Diversely, other studies demonstrate that the maximum production of connective tissue occurs 30 days after microneedling, justifying the use of the GSM-30 group. Thus, the study sought to analyze by comparison the period when collagen production occurred more effectively after microneedling, based on the count of new collagen fibers induced by the technique.

Post-operative care

The wound was cleansed with 0.9% saline solution daily for 7 days in order to prevent possible infections throughout the initial healing process.

Histology

A sample of the skin of each animal was harvested for histological analysis using cold scalpel biopsy, leaving a margin of 1cm between the scar and the biopsy’s incision. The fragments were removed and immediately placed in 10% formalin.

After preparation of the specimens on slides for microscopic study, they were stained with hematoxylin / eosin and Picrosirius, which evidenced the newly synthesized collagen fibers.

Morphological analysis of the samples was performed using optical microscopy by a specialized dermatopathologist. Likewise, the quantification of collagen fibers was carried out by a qualified professional from the Morphofunctional Laboratory of the Universidade do Estado of Pará (UEPA).

The slides for studying the collagen were visualized in a Zeiss microscope with 100x magnification and polarized light. Three photographs were taken of the area of fibrosis of each slide with assistance of the Axion Vision software. The microphotographs were analyzed using the Image J software, aided by the Threshold Color plugin. The percentage of collagen was computed based on the analysis of the colors of particles, selection and measurements of areas. The matrices 0-40 and 45-120 were respectively used for the red (type I collagen) and green (colla-
gen type III) colors, with 0-255 saturation and 5-225 brightness, according to the protocol standardized by Bedoya et al. (2016).\textsuperscript{12}

The microscopic findings were observed and classified regarding the presence or absence of reepithelialization and granulation tissue in a semiquantitative way in the samples as follows 0 - absent, 1 – scarce, 2 – discrete, 3 – moderate, and 4 - marked.\textsuperscript{13}

**STATISTICAL ANALYSIS**

The results of the present study were obtained through statistical analysis using the Fisher’s exact test for qualitative morphological variables (reepithelialization, granulation and inflammation). The quantitative variables were in turn evaluated using the Student’s t-test and Mann-Whitney test, with a significance level of \( p < 0.05 \). The Microsoft Office Excel and Microsoft Office Word softwares (version 2010), were used for preparing tables, graphs and texts.

**RESULTS**

The presence of reepithelialization and absence of granulation tissue were similar in GS and GSM groups – both at 14 and at 30 days after the microneedling procedure – without statistical difference.

The rate of fibrosis was reduced in GSM by 19% on average, as compared to the GC at 14 days, while in GSM-30 that reduction was 4% as compared to GS-30. Nevertheless, both reductions were not statistically significant.

Taking into account the analyzed medians, the concentration of type I collagen increased GSM-14 and in GSM-30 when compared to their respective controls (GS-14 and GS-30), however this increase was not statistically significant. Type III collagen concentration also increased in GSM-14 regarding GS-14 when their medians were taken into account, however this increase was not statistically significant. At the 30-day experimental time point, the median in GSM-30 was lower than in GS-30, with no statistical relevance.

Figures 1 and 2, obtained through histology, demonstrate the proliferation of collagen fibers in GS-30 and GSM-30, respectively. Fibers stained in green represent type III collagen, while those stained in red represent type I collagen. In this manner, it is possible to visualize a larger amount of type I (red) collagen fibers in GSM-30 (Figure 2), corroborating the outcomes found above.

In GSM-14, 75% of the animals had a maximum of 62.1% of type I collagen, whereas in GSM-30 this figure was 64.5%, without statistical difference. Regarding the control groups, 75% of the animals in GS-14 had a maximum of 37.8% of type I collagen, whereas in GS-30 this value was 63.4%.

As for type III collagen, 75% of the animals in GSM-14 had a maximum of 19.8% collagen III, while in GSM-30 this value was equal to 20.8%, with no statistical difference. Regarding the control groups, 75% of the animals in GS-14 had a maximum of 18.4% type III collagen, whereas in GS-30 this value was equal to 13.8%.

**DISCUSSION**

Microneedling is currently emerging as a highly effective alternative for the correction of cutaneous scars as compared to classic ablative treatments, such as chemical peels, dermabrasion and lasers.\textsuperscript{5} Unlike these, the microneedling technique is aimed at stimulating the production of collagen without causing the total removal of the epidermis, having been termed collagen induction therapy (CIT), since the inflicted microtraumas favor the release of chemical mediators, with a view to replace the damaged tissue with scar tissue.\textsuperscript{14}

Among the variables analyzed in the present study, reepithelialization in the scars was taken into account. According to Campos et al.,\textsuperscript{15} reepithelialization occurs early during the healing process, meaning this takes place still in the proliferative

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{GS-30_slide_stained_with_Picrosirius_x50_magnification.png}
\caption{GS-30 slide stained with Picrosirius, x50 magnification}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{GSM-30_slide_stained_with_Picrosirius_x100_magnification.png}
\caption{GSM-30 slide stained with Picrosirius, x100 magnification}
\end{figure}

Figures 3 and 4 represent the proliferation of collagen fibers in GS-14 and GSM-14, respectively, with green fibers and red fibers corresponding to type III and type I collagen.
phase, which comprises the first weeks of the cicatricial mechanism. The findings of the present study are in line with those of Campos et al., since all animals experienced complete reepithelialization of the induced lesion, since the time necessary for the completion of the natural reepithelialization process had already elapsed.

On the other hand, the animals that underwent microneedling had a small loss of integrity of the skin barrier at the time of the procedure, since the technique’s principle consists of puncturing the epidermis without removing it, in order to trigger the cicatricial regeneration process proposed by microneedling. Thus, reepithelialization following the use of microneedles occurs more rapidly (within roughly 5 days), since the epidermis is not totally removed, but rather only perforated.

In light of this, the animals of the control groups (GS-14 and GS-30) experienced reepithelialization of the lesion in the first weeks, while the animals that underwent microneedling (GSM-14 and GSM-30) had a new reepithelialization after this procedure. However, no statistically significant difference was observed within the groups (p = 1), since reepithelialization had already been completed at the time of the histologic analysis due to the fact it is an early mechanism both in physiological healing and after the use of microneedles.

Another variable analyzed was the presence of granulation tissue. The production of this tissue originates at the beginning of the healing process (proliferative phase). It is predominantly composed of fibroblasts, which in turn will give rise to collagen, the main constituent of the final scar. The granulation tissue therefore undergoes a remodeling process during the healing process, meaning that it is progressively replaced by collagen fibers up until the point the latter predominate in the scar.

In this manner, the results obtained in the present study corroborate those of the literature, since all animals did not present granulation tissue at the end of the experiment, thus proving that the healing process had been completed.

Regarding the proportion of fibrosis in the scars analyzed, the groups that underwent microneedling had fibrosis reduction as compared to the control groups. GSM-14 presented a mean reduction of 19% in the proportion of fibrosis regarding the total area of the wound, while in GSM-30 the fibrosis was reduced by 4% on average (Graph 1).

According to Ferguson et al., the TGF-β3 molecule induces a scar regenerative response, whereas TGF-β1 and TGF-β2 induce fibrotic scarring. Despite the fact that the effects of microneedling have not been analyzed in the present study, it can be inferred that the procedure provided reduction of fibrosis, corroborating the research of Aust et al., which proved that the microneedling technique is capable of inducing the expression of TGF-β3 – which is maintained over the following 2 weeks – as well as of decreasing the expression of TGF-β1 and TGF-β2. In light of this, the largest fibrotic reduction in GSM-
14 is justified, since the scars in this group were analyzed exactly 2 weeks after the microneedling procedure.

In addition, the more modest degree of fibrosis reduction in GSM-30 might be justified by fact that a single microneedling session was performed in this study, given that other studies recommend microneedling sessions with monthly intervals for better outcomes. As a conclusion, further research should be performed aimed at assessing the proportion of fibrotic tissue in the scars after monthly microneedling sessions, thus elucidating whether an increase in the number of sessions leads to a more effective reduction of fibrosis.

According to several studies – such as those by Cunha et al., Tizzato et al., and Negrão et al., all of which performed in 2015, as well as that by Palheta et al., performed in 2016 – describe the action of microneedling on the stimulation of the production of collagen fibers and on the reconstruction of cicatricial tissue in the normal skin. Consequently, this technique improves the quality of scars. In this way, this study was expected to lead to outcomes that indicated a greater amount of collagen in the groups that underwent microneedling (GSM-14 and GSM-30).

In this sense, there was a numerical increase of both type III and type I collagens concentrations in GSM-14 as compared to GS-14, which did not undergo the technique. Taking into consideration the median percentage of type I collagen, it is possible to reaffirm the induction of the collagen production capacity generated by the microneedling technique. Although this increase was not statistically significant, there is a tendency to the expected result (Graph 2).

Based on the literature, CIT generates an increase in type I collagen – which is stronger and more resistant – and a decrease in the expression of type III collagen. In face of this, when analyzing the group with the longest experimental duration (GSM-30), type III collagen was lower when compared to the median of the group that did not undergo microneedling (GS-30). This fact suggests that the decrease in type III collagen is seen later on, after the procedure, since after 14 days the group that underwent microneedling (GSM-14) had a greater number of type III collagen fibers as compared to its control (Figure 4), although both groups did not present statistically significant differences (Graph 3).

Important data were derived from the comparative analysis of collagen type I concentration’s percentiles between groups in both periods (Graph 4). The progression in GSM groups (14 and 30 days) revealed high rates of type I collagen as early as 14 days (GSM-14) following the microneedling session when compared to GS-14 (Graph 4), which did not undergo the technique. This number then remains almost constant for up to 30 days (GSM-30). This result is in line with studies that indicate the presence of induction of collagen production by the technique, suggesting promising results already within a period of 14 days after the procedure.

Nevertheless, doubts may arise regarding the number of subsequent sessions, in the optimization of this outcome based on the repetition of sessions for enhancing results within the 30-day post procedure period, as suggested by Zeitter et al. in a recent study. These authors found more effective results with 4 sessions as compared to the group that underwent only 1 session in atrophic scars. Therefore further studies should be carried out aimed at establishing the ideal number of sessions for normotrophic scars.

Regarding type III collagen, a different trend was observed in the comparison of groups. Although GSM-14 and GSM-30 maintained an almost constant pattern, there was a decrease in type III collagen in GS-14 and GS-30. These data
can be attributed to the natural replacement of collagen type III by type I collagen, a phenomenon already described in the literature. Other studies using microneedling with microneedles of various lengths focusing on the evaluation of the frequency of and interval between treatments for enhanced effects would be the next appropriate step to further confirm the effectiveness of the microneedling technique.28

While outcomes in GC14 and GC30 were not constant, it was possible to observe low values of type I collagen in 14 days (GS-14) as well as an exponential increase from 14 to 30 days (GS-30) – which occurs physiologically in the healing process – without, however, statistical significant difference between the groups. It was also observed that at 14 days the group that underwent previous microneedling had greater expression of type I collagen fibers than the one that did not. In contrast, at the end of the 30-day period, both had similar amounts of this collagen, which suggests the necessity of further studies with larger samples and their respective long-term outcomes, aiming at defining the ideal number of sessions.29

CONCLUSION

Therefore, based on the outcomes of the present experimental study, it was possible to conclude that the microneedling technique was proven effective in stimulating collagen fibers in scars resulting from induced lesions. Although there was no statistical difference in the analyzes performed, it can be inferred that there was a tendency for improvement of the scars as a result of the decrease in fibrotic tissue, as well as greater production of collagen fibers, which have been shown to promote scar regeneration caused by the use of microneedles.

Regarding the studied experimental time lapses, it can be inferred that the production of both types of collagen (I and III) is more intense during the 14 day-period following microneedling, as compared to the control, with greater expression of type I collagen fibers. Regarding the 30-day post procedure period, it was observed that the animals that underwent microneedling had an effective replacement of type III collagen with type I collagen – a necessary and beneficial event in the cicatricial regeneration process.

In this manner, microneedling acts by stimulating a greater production of collagen fibers – especially type I – in a shorter period (14 days), and that remain constant on subsequent days. In the 30-day period after the procedure, the replacement of collagen fibers is more effective.

Align with these findings, further experimental studies should be performed aimed at elucidating whether monthly microneedling sessions – rather than a single session – would promote better outcomes, both by stimulating increased production of type I fibers and promoting the effective replacement of type III collagen with type I collagen in the long run.●

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Microneedling on scars from induced cutaneous injuries


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