



Surgical & Cosmetic Dermatology

ISSN: 1984-8773

Sociedade Brasileira de Dermatologia

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Efeitos da andiroba associada ao microagulhamento na pele de ratos  
Surgical & Cosmetic Dermatology, vol. 10, núm. 4, 2018, Outubro-Dezembro, pp. 323-326  
Sociedade Brasileira de Dermatologia

DOI: 10.5935/scd1984-8773.20181041279

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# Effects of *Carapa guyanensis* associated to microneedling in mice skin

Efeitos da andiroba associada ao microagulhamento na pele de ratos

DOI: <http://www.dx.doi.org/10.5935/scd1984-8773.20181041279>

## ABSTRACT

**Introduction:** Microneedling is a technique used to treat scars with increasing popularity in dermatology. In this context, associated to micro-needling, drugs and active substances can be administered through the technique known as drug delivery. We highlight the use of vitamin C, due to its known antioxidant properties. Another example is *Carapa guianensis* Aubl., due to its anti-inflammatory, analgesic, antiseptic and healing properties.

**Objective:** To evaluate the effects of *Carapa guyanensis* associated to microneedling.

**Methods:** Thirty-one male mice, distributed into five groups, were used: GPi (pilot), with three animals; GM (micro-needling); GMA (micro-needling + *Carapa guyanensis*); GMV (microneedling + vitamin C); and GOM (microneedling + mineral oil), with seven animals in each group. The animals were submitted to the microneedling technique on D0 (day 0) and to the application of the substance of its respective group in the skin, in the morning for seven days.

**Results:** There was a significant difference between the expression of fibroblast proliferation and vascularization observed in the tissue of animals in groups GM and GMV. The expression of collagen fibers was superior in animals in the GMV group.

**Conclusions:** *Carapa guyanensis* oil had inferior results as drug delivery when compared to vitamin C.

**Keywords:** Ascorbic acid; Collagen; Rats

## RESUMO

**Introdução:** O microagulhamento é uma técnica utilizada para o tratamento de cicatrizes e apresenta cada vez mais destaque na Dermatologia. Nesse contexto, em associação com o microagulhamento, drogas e ativos podem ser administrados por meio da técnica conhecida como drug delivery. Destaca-se o uso da vitamina C, por sua já comprovada ação antioxidante. Outro exemplo é a andiroba (*Carapa guianensis* Aubl.), por suas propriedades anti-inflamatória, analgésica, antisséptica e cicatrizante.

**Objetivo:** Avaliar os efeitos da andiroba associada ao microagulhamento.

**Métodos:** Foram utilizados 31 ratos machos distribuídos em cinco grupos: GPi (piloto), com três animais; GM (microagulhamento); GMA (microagulhamento + andiroba); GMV (microagulhamento + vitamina C); e GOM (microagulhamento + óleo mineral), com sete animais em cada grupo. Os animais foram submetidos à técnica do microagulhamento no D0 (dia 0) e à aplicação da substância do seu respectivo grupo, na pele, pela manhã, durante sete dias.

**Resultados:** Houve diferença significativa entre a intensidade de proliferação fibroblástica e a vascularização observadas no tecido dos animais dos grupos GM e GMV. Já a intensidade de fibras colágenas foi superior nos animais do grupo GMV. **Conclusões:** O óleo de andiroba como drug delivery obteve resultados inferiores à vitamina C.

**Palavras-Chave:** Ácido ascórbico; Colágeno; Ratos

## INTRODUCTION

Microneedling is a technique used for the treatment of scars that has been increasingly becoming prominent in Dermatology.<sup>1</sup> It consists of using a cylindrical roller made of polyethylene equipped with sterile stainless steel microneedles of between 0.25mm and 2.5mm in length that is repeatedly rolled

## Original Articles

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Received on: 08/11/2018

Approved on: 10/12/2018

This study was performed at the Universidade do Estado do Pará - Belém (PA), Brazil.

Financial support: None.

Conflict of interests: None.



on the skin (10 to 15 times) so that it inflicts micropunctures to it (250 to 300 micropunctures per cm<sup>2</sup>).<sup>2</sup>

These multiple micropunctures cause microhematomas in the dermis, with stimulation of cytokines release (such as IL-1 and TNF- $\alpha$ ), which promote the formation of new collagen in a process called percutaneous collagen induction. Bearing in mind this progression of events, the cicatricial process caused by microneedles can be divided into three phases:

*Injury phase*, in which there is release of platelets and neutrophils that produce cytokines, stimulating keratinocytes and fibroblasts;

*Healing phase*, when the formation of the fibronectin matrix, where the collagen type III will be deposited, occurs;

*Maturation phase*, in which the more resistant type I collagen replaces collagen type III.<sup>3</sup>

In this context, and considering that the trauma inflicted by the microneedles forms conduits in the tissue, drugs and active principles can be administered in association with this technique. This association is also known as drug delivery, with vitamin C – which has an already proven antioxidant action – standing out as an example of drug administered via this method.

In addition, other vitamins, such as vitamins A and B3, as well as copper peptides, zinc, hyaluronic acid and growth factors can also be used.<sup>4</sup> In another study, drug delivery was performed with copaiba extract, leading to higher production of collagen and fibroblasts in the skin of rats.<sup>1</sup>

Also in connection with the drug delivery technique, the extract of andiroba (*Carapa guianensis* Aubl.) is another intensely studied plant-based substance, whose chemical composition contains stearin, fatty, oleic and myristic acids, and, to a lesser extent, palmitic and linoleic acids. Among its properties it is possible to emphasize anti-inflammatory, analgesic, antiseptic and healing actions in addition to effects on the respiratory tract.<sup>3</sup>

Considering the importance of the research on the use of vegetable extracts, as well as the presence of already significant results obtained with the application of such substances to the skin, the present study is aimed at evaluating the effect of microneedling associated to drug delivery of andiroba extract in the skin of rats.<sup>1</sup>

## OBJECTIVES

General objective: to evaluate the effect of microneedling associated with the use of andiroba on rats' skin.

Specific objective: to describe the morphological and morphometric aspects of the effects resulting from the application of the microneedling associated with andiroba on rats' skin.

## METHODS

Thirty-one male Wistar rats, weighing between 220g and 250g, with approximately 120 days of life, were supplied by Instituto Evandro Chagas' breeding vivarium (Belém - PA, Brazil). The animals were kept at a temperature of approximately 22  $\pm$  2 °C, with adequate relative humidity (around 55  $\pm$  10%), artificial luminosity of 12-hour photoperiod (12h illumination/12h dark, with the illumination period taking place from 06:00 to

18:00) and controlled noise. The animals were grouped together according to the study protocol in plastic cages lined with sterile wood shaving, with water and feed offered *ad libitum* throughout the study. The cages were cleaned every other day.

Distribution of the experimental groups: the animals were randomly distributed into five groups:

- Pilot Group (GPi): three animals, the techniques used in this study were practiced in this group;

- Microneedling Group (GM): seven animals that underwent the microneedling technique on D0 (day zero) and euthanasia on D30 (day 30) of the procedure, in order that a tissue sample could be collected for histological evaluation;

- Microneedling + Andiroba Group (GMA): seven animals that underwent microneedling on D0, with application of 1ml andiroba extract in the wound in the morning for seven days, and euthanasia on D30 of the procedure, in order that a tissue sample could be collected for histological evaluation;

- Microneedling + vitamin C Group (GMV): seven animals that underwent microneedling on D0, with application of 1ml liquid vitamin C in the wound in the morning for seven days, and euthanasia on D30 of the procedure, in order that a tissue sample could be collected for histological evaluation;

- Microneedling + Mineral oil Group (GOM): seven animals that underwent microneedling on D0, with application of 1ml mineral oil in the wound in the morning for seven days, and euthanasia on D30 of the procedure, in order that a tissue sample could be collected for histological evaluation. The mineral oil was used as a positive control group due to the fact it has oily constitution, therefore simulating a possible effect of the liquid containing the andiroba extract when applied to the skin. However, vitamin C will be used in order to compare its effects with those of the plant extract, since it is rich in that nutrient.

## Technique

**Microneedling:** The rats were placed in dorsal decubitus position. Trichotomy was performed in a 5x5 cm area in the dorsal region. Microneedling was performed subsequently with the aid of a roller with 0.5 mm microneedles. This procedure was performed with the roller positioned between the index finger and thumb, with moderate force, in one-way direction motions, up until a uniform petechiae pattern had arisen (about 10 times).

**Application of the liquid containing andiroba extract:** In the Microneedling + Andiroba Group (GMA), 1ml of andiroba extract was applied immediately after microneedling, and then in the morning, for 7 days.

**Application of mineral oil:** In the Microneedling + Mineral oil Group (GOM), 1ml of mineral oil was applied immediately after microneedling, and then in the morning, for 7 days.

**Application of vitamin C:** In the Microneedling + vitamin C Group (GMV), 1ml of liquid vitamin C was applied immediately after microneedling, and then in the morning, for 7 days.

**Histological analysis:** The harvested material was conditioned and stained by hematoxylin-eosin and picosirius techniques, for evaluation of collagen fibers. The slides were ana-

lyzed by a pathologist physician using optical microscopy, with observation and classification of the following findings: intensity of collagen fibers, inflammatory infiltrate, and vascular proliferation, according to the following ratings: (0) = absent, 1 = mild, 2 = moderate, and 3 = intense, in addition to the evaluation of the epidermis' thickness ( $\mu\text{m}$ ).

Statistical analysis: The data obtained by the study were stored in an Excel 2010 worksheet for subsequent statistical analysis of multiple comparisons, performed by the ANOVA test, followed by the Student's *t*-test. When there was statistically significant difference, the Tukey test was used with a significance level  $\alpha = 0.05$ . For this purpose, the software Bioestat® 5.3 (Instituto de Desenvolvimento Sustentável Mamirauá, Brazil) was used.

## RESULTS

Regarding the fibroblastic proliferation, the only significant difference found was between the intensity of proliferation observed in the tissue of animals in GM and GMV, corresponding to median levels, classified as 'mild' and 'moderate', respectively ( $p = 0.0127$ ; Figure 2). Similarly, there was significant difference only between these groups when the intensity of vascularization was evaluated, with the GM group presenting a median level classified as 'mild', while the GMV group was a 'moderate' median level ( $p = 0.043$ ).

The intensity of collagen fibers was higher in the animals in GMV when compared to that observed in the tissues of the animals in other study groups, being considered 'intense' in this group (Med = 3), 'mild' in GM and GMA groups (Med = 1), and 'moderate' in GMO (Med = 2) ( $p = 0.006$ , Figure 3).

There were no statistically significant differences in concentrations of collagen types I ( $p = 0.1491$ ) and III ( $p = 0.1777$ ), when compared to a given group.

However, there was a significant difference between densities of type I and III collagens within each group, with type I collagen present in higher concentrations in all the evaluated groups.

## DISCUSSION

In 2014, Henriques described the benefits of andiroba oil in the anti-inflammatory and cicatricial processes of the skin. However, regarding the proliferation of fibroblasts, a satisfactory result was not observed in that study as compared to other substances. This can be explained by the healing stages of the skin.<sup>5</sup>

It is known, therefore, that the microneedling technique generates a cicatrization process with the initial induction of an injury phase that triggers a series of inflammatory events so that, in the second phase (cicatrization phase), the neutrophils are replaced by monocytes and proliferation of fibroblasts, followed by the production of type III collagen. After a few days, the fibronectin matrix is formed, allowing the deposition of collagen just below the basal layer of the epidermis. In the third phase (maturation phase), type III collagen, which is predominant in the early stage of the healing process, is slowly replaced by type I collagen, which is more durable, persisting for years. Therefore,

further studies should be conducted aimed at clarifying whether andiroba oil's anti-inflammatory properties promote some alteration in the healing phases, which would therefore lead to a decrease in the proliferation of fibroblasts and collagen.<sup>3,6</sup>

According to another 2014 study, topical vitamins – such as vitamin C – promote increased connective tissue production and epithelial cells when associated with microneedling. In addition, they are necessary to potentiate the effect of the technique in skin regeneration. That study is in line with the results found, since the group that used vitamin C combined with microneedling was more successful in the intensity of fibroblastic proliferation as compared to the other groups. In this manner, they show that the use of vitamin C will later generate a greater amount of collagen than the other substances tested.<sup>7</sup>

Therefore, microneedling has as its main objective to stimulate the production of collagen fibers. According to Lee, in 2014,<sup>8</sup> and Seo, in 2013,<sup>9</sup> these effects are enhanced and potentiated when the procedure is combined with the topical application of vitamin C. Similar results were found in the present study, since the GMV group obtained an intensity of superior collagen fibers when compared to that of andiroba, which did not show a statistically significant difference when compared to the group that underwent only microneedling. However, this observation is in disagreement with Palheta's 2017 studies,<sup>1</sup> that found a higher production of collagen fibers when the copaiba oil was associated with microneedling. Regarding mineral oil, a greater intensity of fibers can be perceived as compared to situations of andiroba oil use. Therefore, more in-depth studies on the properties of andiroba are needed.<sup>8,9,10</sup>

It is important to note that, regarding the natural healing process, the present study demonstrated the presence of homogeneity in the healing stages of the groups, since all groups were already in the maturation stage, with effective collagen replacement evidenced by the predominance of collagen type I, which was present in all groups as compared to collagen type III.<sup>11</sup>

Regarding neovascularization, a statistically significant difference was observed between the microneedling and vitamin C groups, although all showed some degree of neoangiogenesis. There are no reports of studies in the literature that have used andiroba for this purpose. However, this finding is in line with Silva's 2015 research, which evaluated the andiroba's effect on the healing of colonrraphies, obtaining more significant results in animals in which the plant's extract was used. In addition, in the same study, it was possible to observe a better outcome of the inflammatory process in those animals that received vitamin C during the study period, a finding aligned with the data found in the present study.<sup>11</sup>

Microneedling corresponds to the perforation of the stratum corneum, without causing damage to the epidermis. This process allows the release of growth factors that will encourage the production of collagen and elastin in the papillary dermis. In the present study, the Microneedling + vitamin C Group had a higher dermis thickness as compared to other groups, with the Microneedling + Andiroba Group yielding effects that were different than expected.<sup>12</sup>

The onset instant of the petechiae pattern varies with the thickness of the treated skin and the length of the needle chosen. Therefore, thinner and looser, commonly photoaged skin will present a uniform petechiae pattern earlier than thick, fibrous skin observed in the dorsal region of the animals used in the experiment, for instance. Therefore, the choice of the needle's length depends on the type of skin to be treated and the ultimate goal of the procedure.<sup>2</sup>

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## CONCLUSION

The use of andiroba oil in the microneedling's drug delivery procedure in rat skin led to poorer outcomes when compared to vitamin C. Greater dermal thickness, greater proliferation of collagen fibers and fibroblasts, and better angiogenesis were obtained in the group of animals in which vitamin C was used. ●


## ACKNOWLEDGMENTS

Above all, we thank God in all things, as well as those who contributed directly or indirectly to this project.


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