Effect of *Hevea brasiliensis* latex sap gel on healing of acute skin wounds induced on the back of rats

*Efeito do gel da seiva do látex da Hevea brasiliensis na cicatrização de lesões cutâneas agudas induzidas no dorso de ratos*

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**Abstract**

**Objective**: to evaluate the effect of topical delivery of latex cream-gel in acute cutaneous wounds induced on the back of rats. **Methods**: we subjected sixteen rats to dermo-epidermal excision of a round dorsal skin flap, with 2.5cm diameter. We divided the animals into two groups: Latex Group: application of cream-gel-based latex throughout the wound bed on postoperative days zero, three, six and nine; Control group: no treatment on the wound. Photographs of the lesions were taken on the procedure day and on the 6th and 14th postoperative days, for analyzing the area and the larger diameter of the wound. We carried out euthanasia of all animals on the 14th postoperative day, when we resected he dorsal skin and the underlying muscle layer supporting the wound for histopathological study. **Results**: there was no statistically significant difference in the percentage of wound closure, in the histopathological findings or in the reduction of the area and of the largest diameter of the wounds among the groups studied on the 14th postoperative day. **Conclusion**: according to the experimental conditions in which the study was conducted, latex cream-gel did not interfere in the healing of acute cutaneous wounds in rats.

**Key words**: Wound healing. Latex. Treatment. Skin. Rats.

**Introduction**

Wound healing consists of a coordinated cascade of cellular and molecular events that interact to enable tissue reconstruction. Tissue loss is a triggering factor for wound healing and it initiates a series of steps that overlap from time to time. It includes inflammation, neoformation and tissue remodeling. Immediately after injury, the healing process begins through the action of a series of growth factors, cytokines and substances released from platelets and damaged blood vessels. After blood clots are formed, inflammatory cells invade the tissue and exert protecting functions against contaminating microorganisms. They are also major sources of growth factors and cytokines that trigger the wound healing proliferative phase. Such phase, in turn, begins with the migration and proliferation of keratinocytes at the wound edges, followed by the multiplication of dermal fibroblasts in the vicinities of the damaged tissue. Subsequently, the fibroblasts begin to produce large quantities of extracellular matrix. Still in the proliferative phase, there is formation of granulation tissue, thus named because of the granular characteristic due to the presence of newly formed capillaries that are essential to the healing process. Finally, there is the transition of the granulation tissue into a mature scar, which is characterized by collagen continuous synthesis and degradation. The scar is a mechanically insufficient tissue, which lack epidermal appendages¹.²

Since ancient times, mankind tries to interfere in the tissue repair process. In recent decades, much effort has been made in identifying substances and techniques capable to promote healing to be used in wound management. The search for substances with angiogenic activity has also been intense due to its strong potential for clinical application. However, wound healing still remains a challenging clinical issue³.

The use of natural latex from *Hevea brasiliensis* rubber tree for medicinal purposes is an alternative that links biocompatibility and low cost. Several studies have suggested that latex presents growth factors capable of acting in human tissues by stimulating neovascularization, cell adhesion and the formation of extracellular matrix, although such action mechanisms has not been fully elucidated so far⁴. Rubber tree latex biomembrane was developed by Coutinho-Netto for therapeutic purposes in the Laboratory of Biochemistry at the Medical School, USP, Ribeirão Preto / SP. In 1996, the first study used this material for the reconstruction of experimental defects in the...
esophagus of dogs. It demonstrated its influence on tissue neoformation, suggesting the possibility of its use as a substitute or a trigger to the formation of organs and tissues, although there has been elimination of the material. Subsequent experimental studies have demonstrated the action of the biomembrane favoring the repair of abdominal wall defects in rats, conjunctival reconstruction in rabbits, and pericardium replacement in dogs. The biocompatibility of the biomembrane, necessary for its use as a biomaterial, has been proven by a number of experimental studies. The biomembrane, in the form of dressing, is useful for treating pressure ulcers, promoting rapid debridement, granulogenesis and healing acceleration. Similar effects were observed in chronic flebopathic ulcers.

Subsequent studies showed that the angiogenic and healing acceleration properties are due to the action of a protein substance found in the latex serum fraction. Such discovery stimulated the development of a cream-gel for topical use, which is indicated for the treatment and acceleration of wounds, produced from the base serum containing the bioactive protein fractions added to a cream-gel base.

This study aimed to evaluate the healing effect of latex cream-gel on induced skin wounds in rats.

**METHODS**

This study was performed at the Laboratory of Experimental Surgery at the Medical School, University of Brasilia, Brazil. We adopted the Ethical Principles in Animal Experimentation recommended by the Brazilian College of Animal Experimentation (COBEA - Colégio Brasileiro de Experimentação Animal) affiliated to the International Council for Laboratory Animal Science, and the Brazilian Legislation on Animal Experimentation, Federal Law No. 6638 (1979). The research project was submitted to and approved by the Ethics in Research Committee on Animal Use of the Medical School, University of Brasilia (protocol approval number 52439/2011).

We studied 16 adult, male Wistar rats (Rattus norvegicus albinus, Rodentia mammalia), with mean age of 60 days and weighing between 188 and 386 grams. We established an acclimatization period of seven days before initiating the experiment. The animals remained in their own accommodation, under room temperature and humidity, in circadian cycle (light / dark), with free access to water and specific diet (Purina ® - Labina).

**Experimental design**

We randomly distributed the animals into two groups, with eight animals each: Latex group – application of latex serum in cream-gel base on the wound bed on postoperative days zero, three, six and nine; Control group: no treatment on the wound. We performed euthanasia of all animals on the 14th postoperative day.

**Surgical procedure**

We anesthetized the animals with xylazine hydrochloride at a dose of 10 mg / kg of body weight, combined with ketamine hydrochloride at a dose of 75 mg / kg of body weight, intramuscularly delivered. Once anesthetized, each animal was placed on the surgical board in prone position. After trichotomy, the surgical technique started, with the same standardization for the animals in all groups, as we previously described in another publication. The center of the epilated region was previously marked with a metallic, 2.5 cm diameter dermatological punch, and the excision of the skin was completed with a scalpel (figure 1). Hemostasis was performed by digital compression with gauze. Then, the animals from the latex group received manual application of latex in cream-gel base, in an amount enough to cover the surface of the wound. In the control group, the lesion was induced, followed only by hemostasis.

The latex cream-gel was reapplied on the third, sixth and ninth postoperative days. All animals, including the ones from the control group, were anesthetized on the mentioned days above. On the third and ninth days, the experimental animals were anesthetized for proper application of the gel-cream on the wound, and the control ones so that the animals were exposed to the same stressful situations, except the application of gel-cream.

Neither group received occlusive dressing after the treatments were applied. At the end of the procedures, the animals were put back in their respective cages, in the same preoperative conditions.

**Documentation of the wounds evolution**

Once the animals were fixed on the operating table, the largest and smallest diameters of the wounds were measured with the help of a caliper in order to be...
compared with the standard initial measurement. At that
time, we recorded the wounds with digital photography.
This procedure was performed on the day the surgery was
appointed to happen, and repeated on the 6th and on the
14th postoperative days. The image of the lesion was
transferred to the Image J® software, and after
establishing the periphery by means of the polyline method
demarcation of all points of the injury), the wound image
was analyzed according to area and the largest diameter
parameters.

Material collection for the study
On the 14th postoperative day, the 16 animals
were anesthetized with intramuscular ketamine and
xylazine. Then, a dorsal pad containing the wound and
the underlying muscle layer was excised. The animals
were sacrificed with a lethal dose of thiopental
intraperitoneally delivered at a dose of 25 mg / kg. The
specimens were preserved in formaldehyde for
histopathological study.

Histopathology
Fragments embedded in paraffin were stained
with hematoxylin and eosin and examined under an optical
microscope. We analyzed the amount of collagen,
fibroblasts and mononuclear and polymorphonuclear
infiltrates. These parameters were graded on a 0-3 scale,
indicating, respectively, samples with no, little, moderate
or great amounts of the analyzed variable. Neovessels were
quantified in five high magnification fields. The presence
or absence of reepithelialization, foreign body, abscess and
hair follicles in the scar were also documented.

Statistical analysis
Data were analyzed using the Sigma Stat® 3.5
software. Comparisons of areas and the larger diameters
of the wounds in latex and control groups in each of the
study days were done by One Way Analysis of Variance
(ANOVA). The Fisher’s Exact and Chi-square tests were
used for histological variables. The significance level (p)
used for rejecting the null hypothesis was 0.05.

RESULTS

Measurements of the wounds
when comparing the control and the latex groups
on the day the surgery was performed, on the 6th and on
the 14th postoperative days, the wound area did not show
a statistically significant difference (Table 1).

The percentage of wound closure from day zero
to day six showed no statistically significant difference in
the intergroup comparison (p=0.136). There was, however,
a higher wound closure percentage in the latex group
compared with the control one, 63.1% and 59.5%,
respectively.

Microscopic evaluation
Tables 2, 3 and 4 show the histological intergroup
comparison on the 14th postoperative day, with no
statistically significant difference, though the number of
neovessels in the latex group, observed in higher
magnifications microscopic fields, was higher when
compared with the control group (Figure 2).

DISCUSSION

Several researches have shown that the
biomembrane produced from natural latex of Hevea brasilienisis biocompatible and has angiogenic, cell
adhesion and extracellular matrix formation properties. In
pressure ulcers, the biomembrane facilitated the rapid
debridement of wounds, granulogenesis and complete
healing, producing flat and aesthetic scars. Similar results
were observed in diabetic patients with abnormal wound
healing. When used in patients with chronic venous ulcers,
the biomembrane worked as a wound healing inducing
factor, particularly in the inflammatory phase, confirmed
by the intense exudation and debridement of the lesions,
leading to changes in the chronic venous ulcer
microenvironment.

The preparation of a latex gel containing the
protein fractions responsible for the induction of
angiogenesis corresponds to the biotechnological
enhancement of the research on Hevea brasilienisis natural
latex. The product was obtained by a technique used to
separate protein fractions through a high performance liquid
chromatography, lyophilization and cream-gel formulation.
According to studies conducted by the manufacturer,
the protein fractions show biological activities that stimulate
angiogenesis, fibroblasts cell proliferation, collagen synthesis
and extracellular matrix strengthening and collagenase
inhibition. A study using latex gel in patients with chronic
ulcers was also conducted by the same group with favorable
results.

Table 1 - Areas of the lesions (in cm²) in the latex and control groups.

<table>
<thead>
<tr>
<th>Day of experiment</th>
<th>Control (n=8) Mean ± SD</th>
<th>Latex (n=7) Mean ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.17 ± 0.88</td>
<td>4.44 ± 0.88</td>
<td>0.544</td>
</tr>
<tr>
<td>6</td>
<td>1.69 ± 0.52</td>
<td>1.60 ± 0.44</td>
<td>0.711</td>
</tr>
<tr>
<td>14</td>
<td>0.04 ± 0.04</td>
<td>0.06 ± 0.03</td>
<td>0.083</td>
</tr>
</tbody>
</table>
Penhavel

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In a study assessing the biocompatibility of the biomembrane, Mrué and colleagues assessed the biomaterial-induced healing by using a model of 0.5 cm circular acute skin ulcers induced in rabbits' ears. The group treated with biomembrane showed early epithelialization when compared with the control one, and in the histopathological samples, the presence of organized collagen fibers was evident and presented no sign of fibrosis and neovessels\(^\text{11}\).

We could not prove the effectiveness of latex gel as for wound area reduction and histopathological findings, since these parameters were not statistically significant when compared with the control group. In the study by Mrué\(^\text{11}\), the biomembrane flexible conformation allowed direct and permanent contact of the ulcer by means of stitches. In a study evaluating angiogenesis, vascular permeability and healing, the latex serum added to a carboxymethylcellulose gel was applied on the day of surgery and on the 3\(^{rd}\), 6\(^{th}\) and 9\(^{th}\) postoperative days, showing accelerated healing\(^\text{16}\). The current study used latex with the same application frequency. However, unlike the above mentioned study, the ulcers have not received occlusive dressing after each application, which may have caused the product to stay less time in contact with the wound. These facts may have interfered in the observation of any difference between this and the other groups. Another limitation of this study is the sample size, with only eight animals in each group. A larger sample could increase its statistical power.

For further studies, we suggest that different quantities of the product should be used, in order to

**Table 2** - Histological comparison between the control and the latex groups on the 14\(^{th}\) postoperative day.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=8)</th>
<th>Latex (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Max/min</td>
</tr>
<tr>
<td>Collagen</td>
<td>2.0</td>
<td>2/2</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>3.0</td>
<td>3/3</td>
</tr>
<tr>
<td>Mononuclear</td>
<td>2.0</td>
<td>2/2</td>
</tr>
<tr>
<td>Polymorphonuclear</td>
<td>2.0</td>
<td>2/2</td>
</tr>
<tr>
<td>Epithelial hyperplasia</td>
<td>1.4</td>
<td>2/1</td>
</tr>
</tbody>
</table>

* Latex x control p=0.234

**Table 3** - Histological comparison between the control and the latex group in the 14th postoperative day (2).

<table>
<thead>
<tr>
<th></th>
<th>Control (n=8)</th>
<th>Latex (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Hair follicle</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Abscess</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Foreign body</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Epithelialization</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

**Table 4** - Comparison between the control and the latex group – quantification of neovessels on the 14\(^{th}\) postoperative day.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=8) Mean ± SD</th>
<th>Latex (n=8) Mean ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessels /field</td>
<td>24.50 ± 9.24</td>
<td>26.25 ± 12.41</td>
<td>0.561</td>
</tr>
</tbody>
</table>

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CONCLUSION

According to the experimental conditions in which the study was conducted, the latex cream-gel did not influence the healing of acute cutaneous wounds in rats.

REFERENCES

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