

## Wound healing using ionic silver dressing and noncrystalline silver dressing in rats<sup>1</sup>

Cicatrização de feridas em ratos utilizando curativos com prata iônica e nanocrystalina<sup>1</sup>

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### ABSTRACT

**PURPOSE:** To investigate the results of the healing process on surgical wounds in the back of Wistar rats using nanocrystalline and ionic silver dressing.

**METHODS:** Sixty rats Wistar were submitted to surgical wounds with punch of 8mm in diameter. In 30 animals (groups PN - nanocrystalline and AD - control) two surgical wounds were done diametrically opposite on the upper back side. On the right side was used nanocrystalline (PN) silver dressing and on the left side, distilled water dressing (AD). On the other group of 30 rats, only one wound was made with the punch, on the right side, and was used ionic silver dressing. So, the groups were divided into three subgroups, according to the day of death (7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day). In each of these days the wounds diameter were measured to evaluate the wound contraction. Microscopic data were analyzed using the H&E staining to verify the inflammatory process and neovascularization. The Masson trichrome staining was used to verify the fibrosis.

**RESULTS:** Macroscopically only the subgroup of 21<sup>st</sup> day showed statistical significance; between the groups AD and PI inflammatory process appeared in the 7<sup>th</sup> day subgroup in 90% of the cases. In neovascularization there was statistical significance between the groups PN and AD in the subgroup of 7<sup>th</sup> day. Fibrosis did not show statistical significance in the studied groups.

**CONCLUSIONS:** In relation to wound contraction, PN and PI groups showed better results than the AD group. In regard to histological analysis, H&E staining showed that there was presence of inflammation in all groups, and at the end, the control group (AD) on 7<sup>th</sup> day, was superior to PN and PI groups. In relationship to fibrosis, no differences were obtained among groups.

**Key words:** Wound Healing. Skin. Silver. Bandages. Rats.

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### RESUMO

**OBJETIVO:** Investigar os resultados da cicatrização de feridas cirúrgicas em dorso de ratos, utilizando curativos de prata nanocrystalina e iônica.

**MÉTODOS:** Sessenta ratos Wistar foram submetidos à feridas cirúrgicas com punch de 8mm de diâmetro. Foram confeccionadas duas lesões diametralmente opostas nos animais dos grupos prata nanocrystalina (PN) e controle água destilada (AD). Na lesão do lado direito foi utilizado curativo com prata nanocrystalina e na do lado esquerdo curativo com água destilada. No outro grupo de 30 ratos, apenas foi realizada uma lesão com o punch no lado esquerdo e curativo com prata iônica. Os grupos foram divididos em subgrupos conforme o dia da morte (7<sup>o</sup>, 14<sup>o</sup> e 21<sup>o</sup> dias), o que caracterizou a existência de três subgrupos, nos quais foram tomadas as medidas do centro das lesões para estudar macroscopicamente a contração da ferida. Microscopicamente foi utilizada a coloração H&E, através da qual foi observado

o processo inflamatório e a neovascularização. Com a coloração Tricômio de Masson, foi estudada a fibrose.

**RESULTADOS:** Macroscopicamente apenas o subgrupo 21 dias apresentou significância estatística entre os grupos AD e prata iônica (PI), porém quando comparados os dias de avaliação, dois a dois, dentro de cada tratamento, todos os subgrupos apresentaram significância estatística ( $p < 0,05$ ). A variável intensidade da inflamação apresentou-se de forma acentuada no subgrupo sete dias em 90% dos casos, entre os grupos AD e PI. Na variável neovascularização houve significância estatística entre os grupos PN e AD no subgrupo sete dias. A variável fibrose não apresentou significância estatística nos subgrupos estudados.

**CONCLUSÕES:** Em relação à contração da ferida, os grupos PN e PI apresentaram resultados superiores ao grupo AD. Com relação à análise histológica, na coloração H&E observou-se que houve presença de processo inflamatório nos grupos estudados sendo que, ao final, o grupo controle (AD) no 7º dia mostrou-se superior aos grupos PN e PI. Com relação à variável fibrose, não houve diferenças entre os grupos.

**Descritores:** Cicatrização. Pele. Prata. Bandagens. Ratos.

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## Introduction

Tissue loss can reach the dermis partial or fully and also include the subcutaneous tissue. Based on this, Mandelbaum *et al.*<sup>1</sup> defined two types of wounds: partial thickness and full thickness. When the wound is in partial, to it is given the name of the dermis incomplete, and the repair is made by reepithelialization of the epithelium or epithelium derived from the unaffected adjacent skin. The final result is almost imperceptible scar. When the wound is of full thickness, dermis is said to be fully extended or in the subcutaneous tissue. There is the need to form a new tissue, granulation tissue, when the epithelization is the basis of healing of partial thickness wounds<sup>2</sup>.

The healing also depends on many local and general factors, as anatomical location, skin type, race and surgical technique<sup>3</sup>. Different classifications are used to facilitate the understanding of the dynamic process and stages. The healing process is divided into five main phases: coagulation, inflammation, proliferation, wound contraction and remodeling.

During the formation of granulation tissue fibroblasts and endothelial cells proliferate and move into the wound, producing extracellular matrix, connective tissue fibers and neovascularization<sup>4,5</sup>. Many fibroblasts acquire some morphological and biochemical properties of smooth muscle cells, giving rise to myofibroblasts. This factor participate in extracellular matrix synthesis and production of mechanical power, affecting the reorganization of the matrix and wound contraction.

The products for wound treatment can be divided into two major groups: topical agents and dressings. The topical agents are those applied directly to the wound bed, or intended to clean or protect the area around<sup>6</sup>. Dressing, also called coverage, is the feature that covers a wound, in order to protect it from external aggression, keeping it moist and preserving the integrity of its region.

Silver has medicinal properties and has been used for over 2000 years. It is a metal that is mostly a byproduct of the mining of lead and is often associated with copper. The antimicrobial property of silver and its compounds is the main base of its medical application since the nineteenth century. Since silver has many uses and has great potential for the treatment of injuries.

The popularity of the use of coatings with silver in wound has been progressively increasing<sup>7,8</sup>. Currently, it is present in a wide range of coverages and can present in two forms: 1) compounds / complexes when associated with a salt and produces the ionic silver (Ag<sup>+</sup>) when in contact with wound fluids or solutions; and 2) elementary silver in metallic form (Ag), also described as colloidal silver or silver nanoparticles.

The nanocrystalline silver was introduced as dressing wounds in 1998 with the claim that it reduces the occurrence of infection and provides opportunity to improve clinical practice in the treatment of wounds<sup>9</sup>.

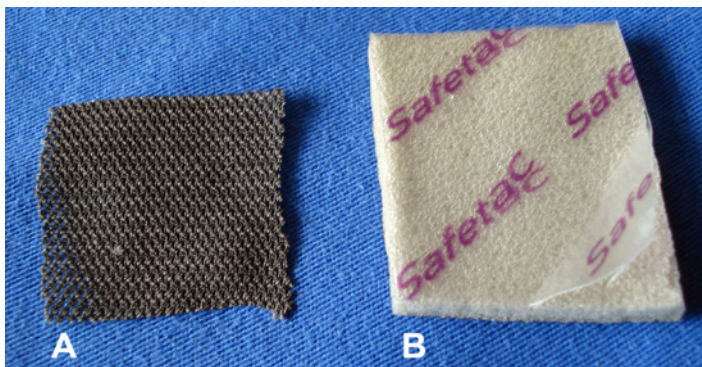
This study aims comparatively evaluate the results of the process of wound healing with the use of nanocrystalline silver dressing with ionic silver and in the dorsal region of rats.

## Methods

This research was accomplished on Medical Research Institute (IPEM) of the Post-Graduate Program in Principles of Surgery of Evangelical Faculty of Parana/Evangelical Hospital of Curitiba and was approved by the Ethics Committee in Research of Beneficent Evangelical Society of Curitiba, PR, Brazil.

Occlusive dressings were used consisting of: 1) absorbing foam, soft silicone (Safetac<sup>®</sup>) and vapor permeable film impregnated with ionic silver coated (Mepilex<sup>®</sup> Ag) forming one group called Silver Ion (PI); 2) coated polyester mesh with nanocrystalline silver (Acticoat 7 Flex<sup>®</sup>), elastic adhesive bandage traditional BSN Medical GmbH group forming nanocrystalline

silver (PN); and 3) sterile distilled water dressing control group, called distilled water group (AD) (Figure 1).



**FIGURE 1** – Dressing with nanocrystalline silver in A and ionic silver in B.

Sixty male Wistar rats were used with age between 110 and 130 days and weight between 220 and 250g. The animals were submitted to general anesthesia with intramuscular administration of Ketamine in dose of 50mg/kg and Xylazine 2% in dose of 10mg/kg. Each animal was positioned in ventral decubitus, trichotomized submitted to antisepsis with alcoholic clorexedine. Were performed two injuries in animals in groups AD and PN (30 rats) (Figure 2) with punch of 8mm, diametrically opposite to dorsal line of the animals. In right side was utilized dressing with silver nanocrystalline (PN) and on left, dressing with gauze soaked in sterile distilled water (AD - control). On 3<sup>rd</sup> group (30 rats) was only made a lesion with the punch on right side, and used over it dressing with silver ion (PI).



**FIGURE 2** - Sample of two injuries with punch of 8mm.

The dressings were changed each seven days, with sedation. The wounds were cleaned with distilled sterile a water and then, each lesion received the connected dressing group.

On death day the animals were subdivided into three subgroups, namely 7<sup>o</sup>, 14<sup>o</sup> and 21 days. They died using solution of Thiopental®, in concentration of 1g diluted in 40ml

of physiological solution, intraperitoneally. Wound diameter measurements were done with graduate scale in cm (Figure 3) and scalpel blade 20 did skin excision at previously demarcated areas (1.5cm out of injuries) (Figure 4). The specimens were preserved in bottles containing 10% formol solution for histology.



**FIGURE 3** - Wound diameter measurements with graduate scale (cm).



**FIGURE 4** - Demarcated areas for skin incision.

Macroscopically in each death day were taken the measurements on center of lesion to analyze the contraction of wound, annotated in a protocol for posterior evaluation.

Microscopically, the results were analyzed using H&E staining to check the inflammatory process and neovascularization, and Masson for fibrosis.

Statistically, were considered the exact Fisher test (independent group) or the binomial test (paired group). Comparisons in relationship the quantitative variables were made considering the nonparametric Mann-Whitney test (independent group) or Wilcoxon (paired group). Values of  $p \leq 0.05$  indicated significance. The data were analyzed with Statistical v.8.0 program.

**Results**

The diameter of injury, when compared the types of treatment, were significant ( $p < 0.05$ ) in 7<sup>th</sup> and 21<sup>st</sup> days between groups PNxAD and ADxPI (Table 1).

**TABLE 1** - Macroscopic evaluation from center of lesion (cm).

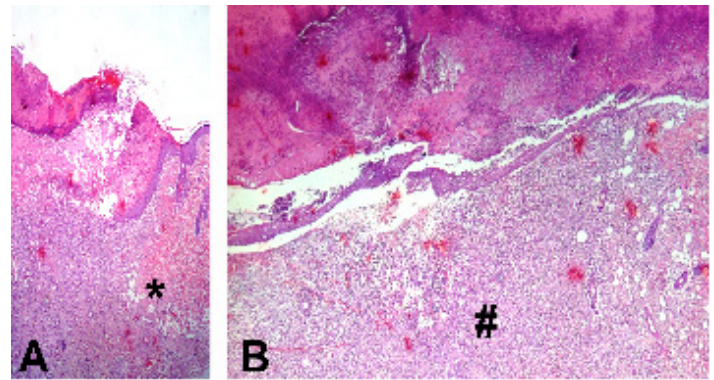
	Subgroups	Group		
		PN (N = 10)	AD (N = 10)	PI (N = 10)
<b>Average</b>	7 days	4.80	5.90	4.80
	14 days	3.40	3.90	3.50
	21 days	0.90	1.00	2.50
<b>Median</b>	7 days	5.00	6.00	5.00
	14 days	3.00	4.00	3.50
	21 days	1.00	2.50	1.00
<b>Minimum</b>	7 days	4.00	5.00	4.00
	14 days	3.00	3.00	3.00
	21 days	0.00	2.00	0.00
<b>Maximum</b>	7 days	6.00	7.00	6.00
	14 days	4.00	5.00	4.00
	21 days	2.00	3.00	2.00
<b>Standard deviation</b>	7 days	0.79	0.74	0.79
	14 days	0.52	0.88	0.53
	21 days	0.74	0.53	0.67
<b>Types of treatment beneath comparison</b>	Subgroups	PN x AD	PN x PI	AD x PI
	7 days	<b>0.038</b>	1	<b>0.011</b>
<b>p value</b>	14 days	0.173	0.739	0.353
	21 days	<b>0.012</b>	0.796	<b>&lt;0.001</b>

When compared the days of evaluation between two groups within each treatment, all groups presented significance ( $p < 0.05$ ) (Table 2).

**TABLE 2** - Diameter of injury comparison between the days of evaluation, two by two within each treatment.

Treatment	7 x 14 days	p value	
		7 x 21 days	14 x 21 days
PN	0.001	<0.001	<0.001
AD	<0.001	<0.001	0.002
PI	0.002	<0.001	<0.001

Intensity of inflammation was more pronounced on group AD on subgroup 7<sup>th</sup> day (Figure 5A and B, Table 3); there was statistic significance only between the groups ADxPI on 7<sup>th</sup> days.



**FIGURE 5** – Photomicrography: A) acute inflammation and epithelium ulceration(\*); B) acute inflammation (#).

**TABLE 3** - Evaluation of inflammatory process with H&E staining.

Intensity of inflammation	Subgroups	Groups		
		PN	AD	PI
<b>Discrete the moderate</b>	7 days	40.00%	10.00%	80.00%
	14 days	100.00%	100.00%	100.00%
	21 days	100.00%	100.00%	100.00%
<b>Accentuated</b>	7 days	60.00%	90.00%	20.00%
	14 days	0.00%	0.00%	0.00%
	21 days	0.00%	0.00%	0.00%
<b>Types of treatment beneath comparison</b>	Subgroups	PN x AD	PN x PI	AD x PI
	7 days	0.375	0.170	<b>0.006</b>
<b>p value</b>	14 days	-	1	1
	21 days	-	1	1

In relationship to days of evaluation, two by two within each treatment, the three groups showed significance when compared the days 7x21 (Table 4).

**TABLE 4** - Comparison of inflammatory process between the days of evaluation, two by two within each treatment.

Treatment	7 x 14 days	p value	
		7 x 21 days	14 x 21 days
PN	<b>0.011</b>	<b>0.011</b>	1
AD	<b>&lt;0.001</b>	<b>&lt;0.001</b>	1
PI	0.474	0.474	1

In microscopic evaluation of neovascularization only had significance on 7<sup>th</sup> day in relationship to groups PN and AD (Table 5).

**TABLE 5** - Assessment of neovascularization between the groups PN, AD and PI.

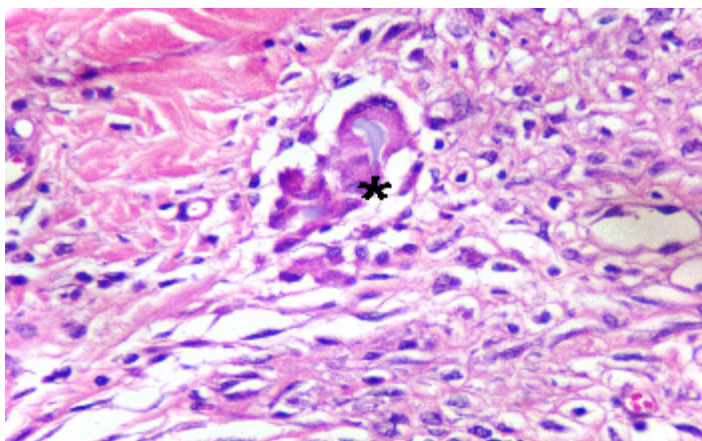
Neovascularization	Subgroups	Groups		
		PN	AD	PI
Discrete / moderate	7 days	70.00%	0.00%	40.00%
	14 days	90.00%	100.00%	80.00%
	21 days	100.00%	77.78%	80.00%
Sharp / intense	7 days	30.00%	100.00%	60.00%
	14 days	10.00%	0.00%	20.00%
	21 days	0.00%	22.22%	20.00%
Types of treatment beneath comparison		PN x AD	PN x PI	AD x PI
	7 days	<b>0.016</b>	0.370	0.087
	14 days	-	1	0.474
	21 days	0.500	0.474	1
p value	7 days	<b>0.016</b>	0.370	0.087
	14 days	-	1	0.474
	21 days	0.500	0.474	1

Also in neovascularization, there was statistic significance ( $p < 0.05$ ) only on group AD when compared the days of evaluation, two by two within each treatment, between the days 7x14 and 7x21 (Table 6).

**TABLE 6** - Comparison between the days of evaluation, two by two within of each treatment.

Treatment	p value		
	7 x 14 days	7 x 21 days	14 x 21 days
PN	0.582	0.210	1
AD	<b>&lt;0.001</b>	<b>0.001</b>	0.210
PI	0.170	0.170	1

Other important finding on microscopy was the presence of macrophages responsible by gigantocellular reaction, that were observed in PI and PN at 7<sup>th</sup> and 14<sup>th</sup> days (Figure 6).

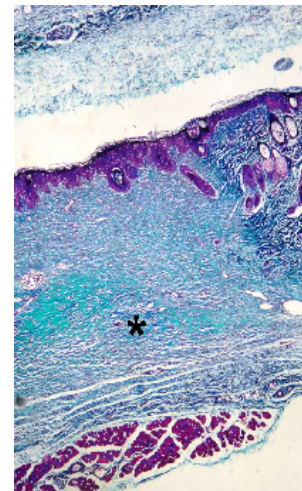


**FIGURE 6** - Photomicrograph showing foreign body reaction(\*).

The lymphocytes appeared on group PN on 14<sup>th</sup> day (two cases) and on group PI on 7<sup>th</sup> day (two cases) and one case on 14<sup>th</sup>

day; however, on control group it did not appear.

In fibrosis evaluation through Masson staining (Figure 7), there was no difference statistically significant between the groups (Table 7).



**FIGURE 7** - Photomicrograph of fibrosis by Masson staining, showing pronounced fibrosis (\*).

**TABLE 7** - Fibrosis analysis by Masson staining.

Fibrosis	Subgroups	Groups		
		PN	AD	PI
Discrete / moderate	7 days	50.00%	0.00%	40.00%
	14 days	70.00%	30.00%	50.00%
	21 days	44.44%	77.78%	40.00%
Sharp / intense	7 days	50.00%	100.00%	60.00%
	14 days	30.00%	70.00%	50.00%
	21 days	55.56%	22.22%	60.00%
Types of treatment beneath comparison		PN x AD	PN x PI	AD x PI
	7 days	0.062	1	0.087
	14 days	0.219	0.650	0.650
	21 days	0.375	1	0.170
p value	7 days	0.062	1	0.087
	14 days	0.219	0.650	0.650
	21 days	0.375	1	0.170

However, in fibrosis analysis comparing the days of evaluation, two by two within each treatment, there was significance on group AD between days 7x21 (Table 8).

**TABLE 8** - Comparison between the days of evaluation of fibrosis, two by two within each treatment.

Treatment	p value		
	7 x 14 days	7 x 21 days	14 x 21 days
PN	0.650	1	0.370
AD	0.211	<b>0.001</b>	0.070
PI	1	1	1

## Discussion

Contrary to Leaper *et al.*<sup>10</sup> that claimed to be irrational the use of silver dressings in non infected wounds, this study proved that there was improvement in healing in groups PI and PN, when compared to the control group (AD).

The macroscopic evaluation consisted on verification of contraction of wound in relation to the demarcated area in surgical procedure day, strategy also used by Araujo<sup>11</sup>.

This verification needs a time since the process of contraction of wound - fourth phase of healing process - consists on centripetal movement of the edges<sup>1,12</sup>. The phase that precedes the wound contraction is the proliferation, responsible for the "closing" of lesion properly said, and is divided in three subphases - reepithelialization, fibroplasia and angiogenesis. At the end, the remodeling phase follows the contraction, the last moment of wound healing. In this research only on subgroup 21 days appeared significant differences between the groups AD and PI, but when compared the days of evaluation two by two within each treatment, all the comparisons between the subgroups indicated significance, more pronounced in comparison between 7x21 days.

In microscopic evaluation with H&E staining - intensity of inflammation -, was found more pronounced form only on seventh day between the groups AD and PI; in group control it appeared in 90% of the cases; however, when are compared the days of evaluation, two by two within each group of treatment, the groups control and PN showed values statistically significant, like findings reported by Pundek *et al.*<sup>13</sup>.

Other important observation was the presence of macrophages responsible by gigantocellular reaction<sup>1</sup>. These cells appear in wound in about one week and within this period in this study were observed two cases in groups PI and PN; also, another two were noticed in the same groups in two weeks. However, control group (AD) had no case in the same time.

The neovessel formation begins with tissue aggression, activation of macrophages and substances produced at this moment. The proteases (plasmin and collagenases) digest the basal membrane and allow that the endothelial cells, stimulated by angiogenic cytokines, form a new vascular site that invades the wound; this is essential to supply oxygen and nutrients for the healing. The angiogenesis ceases by apoptosis<sup>14,15</sup>. In this research, the comparison PN and AD on first sacrifice (7<sup>th</sup> day) presented significance, as well as control group when compared the days of evaluation, two by two within each treatment; this fact was also related by Balbino *et al.*<sup>16</sup>.

Another technique of coloring used was the Masson

staining to observe the formation of collagen (fibrosis). The formation of tissue of granulation depends of fibroblasts, critical cell in matrix formation, in production of elastin, fibronectin, glicosaminoglicans and proteases, all responsible for physiological debridement and remodeling of injury. Within this analysis only the control group presented statistic significance in comparison between the days of evaluation, two by two within each treatment.

## Conclusions

In relation to wound contraction, nanocristaline (PN) and ionic silver (PI) groups showed better results than the AD group. In regard to histological analysis, H&E staining demonstrated that there was presence of inflammation in all groups, and at the end, the control group (water dressing) on 7<sup>th</sup> day, was superior to PN and PI groups. In relationship to fibrosis, no differences were obtained among groups.

## References

1. Mandelbaum SH, Santis EP. Cicatrização: conceitos atuais e recursos auxiliares. *An Bras Dermatol.* 2003;78(4):393-410.
2. Forrest RD. Início da história de tratamento de feridas. *J Soc Med.* 1982;75(3):198-205.
3. Levenson SM, Crowley LV. The healing of rat skin wound. *Ann Surg.* 1965;64(1):288-94.
4. Sampaio CPP, Biondo-Simões MLP, Trindade LCT, Farias RE, Pierin RJ, Martins RC. Alterações inflamatórias provocadas pelo metronidazol em feridas: estudo experimental em ratos. *J Vasc Bras.* 2009;8(3):232-7.
5. Abercrombie M, Flint MH. Collagen formation and wound contraction during repair of small excised wounds in the skin of rats. *J Embryol Exp Morphol.* 1954;2(3):264-74.
6. Clore JN, Cohen IK, Diegelmann RF. Quantitation of collagen types I and III during wound healing in rat skin. *Proc Soc Exp Biol Med.* 1979;161(3):337-40.
7. White RJ. An historical overview of the use of silver in wound management. *Br J Community Nurs.* 2001;6(8)(Silver Suppl 1):3-8.
8. Systagenix. A prata aplicada ao tratamento de feridas. Disponível em [www.systagenix.com.br/cms/uploads/Boletim\\_da\\_Prata.pdf](http://www.systagenix.com.br/cms/uploads/Boletim_da_Prata.pdf).
9. Burrell RE, Wright JB, Hegggers JG, Davis GJ. Efficacy of silver coated dressings as barriers in a rodent burn sepsis model. *Wounds.* 1999;11(4):64-7.
10. Leaper DJ. Silver dressing: their role in wound management. *Int Wound J.* 2006;3:282-94.
11. Araujo URMF, Czezczko NG, Ribas-Filho JM, Malafaia O, Budel VM, Balderrama CMSR, Zimmermann E, Dietz UA. Reparo intraperitoneal de defeitos da parede ventral do abdome com telas de poliéster com colágeno e polipropileno com ácido poliglicólico. *Rev Col Bras Cir.* 2009;36(3): 241-9.
12. Huang Y, Li X, Liao Z, Zhang G, Liu Q, Tang J, Peng Y, Liu X, Luo Q. A randomized comparative trial between Acticoat and SD-Ag in the treatment of residual burn wounds, including safety analysis. *Burns.* 2007;33(2):161-6.
13. Pundek MRZ, Czezczko NG, Yamamoto CT, Pizzatto RF, Czezczko LEA, Dietz UA, Malafaia O. Estudo das telas cirúrgicas de

polipropileno/poliglicaprone e de polipropileno/polidioxanona/  
celulose oxidada regenerada na cicatrização de defeito produzido na  
parede abdominal de ratos. ABCD Arq Bras Cir Dig. 2010;23(2):94-  
9.

14. Hanson D, Langemo D, Thompson P, Anderson J, Hunter S. Understanding wound fluid and the phases of healing. *Adv Skin Wound Care*. 2005; 18(7):360-2.
15. Singer MJ, Cabral ACV, Boyaciyan K, Kulay Júnior L. Aspectos ultraestruturais dos fibroblastos e dos macrófagos durante o processo de reparação da pele de ratos. *Rev Paul Med*. 1986;104(3): 132-5.
16. Balbino CA, Pereira LM, Curi R. Mecanismos envolvidos na cicatrização: uma revisão. *Rev Bras Cienc Farm*. 2005;41(1):27-51.

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