ORIGINAL ARTICLE

ORGANIC SILICON IN THE ACCELERATION OF TISSUE HEALING IN WOUND HEALING BY SECONDARY INTENTION

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Highlights: (1) 15% organic silicon accelerates wound healing in secondary intention lesions. (2) Treatment with organic silicon showed no hematological, metabolic, hepatic, or renal toxicity. (3) Organic silicon did not affect food and water intake or body weight, thereby indicating safety and tolerability.

PRE-PROOF
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ABSTRACT

Objective: To evaluate the efficacy and safety of different concentrations of organic silicon in tissue repair in wounds with healing by second intention. **Methodology:** A total of 27 Wistar rats were divided into five groups: Control (n=5), Hydrogel (n=5), 5% Silicon (n=5), 10% Silicon (n=6), and 15% Silicon (n=5). For this purpose, incision and dissection of the tissue were performed in the region of the dorsal midline close to the scapulae, up to the muscular fascia (2 cm² area). Following this, the wound was cleaned daily with saline and gauze in all animals, with subsequent treatment with organic silicon at concentrations of 5%, 10%, and 15%, and hydrogel, respectively. At the end of 23 days, euthanasia and blood collection for hematological and biochemical analysis were performed. **Results:** Treatment with 15% organic silicon was more effective in shedding the crust and in tissue repair of the lesion. The safety of silicon in the treatment of wounds was demonstrated from laboratory tests that did not indicate hematological, hepatic, and renal toxicity with the proposed treatment. **Conclusion:** It was found that 15% organic silicon is effective in accelerating wound healing by second intention and has not shown any toxic effect on hematological, metabolic, hepatic, and renal parameters.
Keywords: Silicon; Wound Healing; Rats Wistar; Treatment.

INTRODUCTION

Epithelial skin lesions can result in wounds that compromise its primary barrier functions and overall health maintenance in the human body. Regardless of their classification level (extent of skin damage), such wounds always raise concern among healthcare professionals[1]. There is a well-established relationship between impaired tissue healing and the severity of health status during patient recovery, which makes individuals more susceptible to

clinical deterioration, such as severe bacterial coinfections[2], as well as impaired thermoregulation [3]. Wound healing and recovery involve a variety of local and systemic factors, including wound size, location, presence of contaminants, patient comorbidities, and nutritional status [4].

Wounds that heal by secondary intention are the most common skin lesions observed in clinical practice, both in hospitals and primary healthcare units. These wounds are characterized by significant tissue destruction, which makes suturing impossible, with healing occurring through tissue contraction until complete closure is achieved. This type of lesion also increases susceptibility to infectious processes, thereby delaying and impairing wound healing [5].

Treatments for wound healing by secondary intention include exudate control, hydration of viable tissue, and support for autolytic debridement, with dressings such as hydrocolloid, hydrofiber, and calcium alginate[6]. However, these dressings are often associated with high costs[7], and there remains a need for innovative coverings that promote faster healing while reducing the risk of secondary infections. Skin is composed of different macromolecules, with its structural framework notably formed by protein fibers. In this regard, researchers have emphasized the importance of preserving tissue fibers to restore skin integrity[8].

Accordingly, studies have been proposed in the search for new compounds to be used in wound healing treatments, one of which is organic silicon. Organic silicon is a transition element derived from silica, one of the most abundant minerals in the Earth's crust. In the human body, however, it is found in the form of esters derived from silicic acid, being a component of bones, blood vessels, cartilage, and tendons. In the field of aesthetics, organic silicon has already been used to stimulate collagen production and promote skin regeneration[9].

Pharmacological interventions may induce toxicity in the body, even when administered topically, as in the treatment for wound healing by secondary intention, and may also exert systemic effects[10]. This has been demonstrated in experimental models by hematological changes[11], biochemical alterations (markers of renal and hepatic lesion)[12], as well as changes in water and food intake and body weight[13].

Therefore, the objective of this study was to evaluate the efficacy and safety of different concentrations of organic silicon in wound healing by secondary intention.

MATERIAL AND METHODOLOGY

Study Design

This was a randomized, prospective, in vivo experimental animal study. The study was divided into three stages: the first involved the preparation of the organic silicon products; the second, the surgical procedure; and the third, the treatment of the animals. After 23 days of follow-up, the animals were euthanized for biological sample and tissue collection.

Ethical Aspects

This study complied with the recommendations of the Brazilian College of Animal Experimentation (CONCEA) and was approved by the Animal Ethics Committee of UNIJUÍ (Protocol nº 005/20, approval date: July 3, 2020).

Study Site

The study was conducted at the Biological Testing Laboratory and the Animal Facility of the Regional University of Northwestern Rio Grande do Sul – UNIJUÍ, under authorization from the local supervisors.

Animals

A total of 27 adult male Wistar rats (Rattus norvegicus albinus), 36 weeks old, with a mean weight of 487.56 ± 52.35 g, were obtained from the UNIJUÍ animal facility. The animals were housed in cages lined with wood shavings, with 2–3 rats per cage, under a 12-hour light/dark cycle, at a controlled room temperature of 22 ± 2°C, with free access to food and water.

Experimental Groups

The animals were randomly assigned into five groups: Control (n=5), Hydrogel (n=5), 5% Silicon (n=5), 10% Silicon (n=6), and 15% Silicon (n=5). Organic silicon is already used in the cosmetics industry, demonstrating efficacy in improving skin texture at concentrations of 5% [14] and 8%. Based on this, we tested 5% and higher concentrations of 10% and 15% to evaluate their effect on wound healing at doses comparable to those of commercial aesthetic products. Helianto Hydrogel, a non-sterile product (ANVISA registration nº 80225200017), was used as the reference treatment for second-intention wound healing.

Food and Water Intake

Food and water consumption were measured every three days. The average intake was estimated by the relationship between supply and consumption: [(total offered per cage – total remaining per cage)/number of animals per cage][15].

Stage 1

Formulations of organic silicon products were prepared at concentrations of 5%, 10%, and 15% for use in wound treatment after surgery.

Stage 2

The surgical procedure to create the cutaneous wound was performed at a veterinary hospital by a licensed veterinarian. Anesthesia was induced with pre-anesthetic medication consisting of pethidine (15 mg/kg, intraperitoneal). After 10 minutes, anesthesia was maintained with isoflurane administered via face mask, diluted in 100% oxygen. The dorsal region was shaved and disinfected with 2% chlorhexidine scrub followed by 0.5% alcohol-based chlorhexidine. During anesthesia, heart rate was monitored by Doppler, while respiratory rate was monitored by thoracic movements.

The wound was created near the scapula (dorsal midline), with the animal placed in the prone position. A 2 cm incision was made using a scalpel (blade number 11) and Metzenbaum scissors (Figure 1). At the end of the procedure, meloxicam was administered at 2 mg/kg

subcutaneously, with a repeat dose 24 hours after surgery. Each animal was identified on the tail using a dermographic pen.

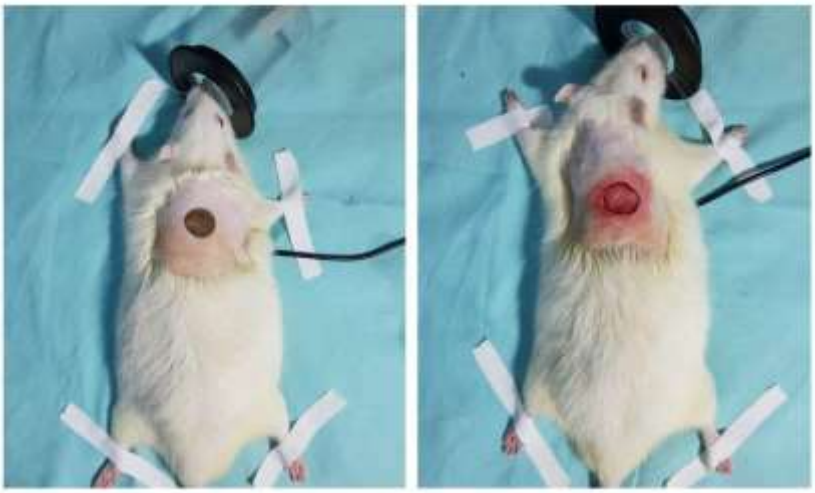
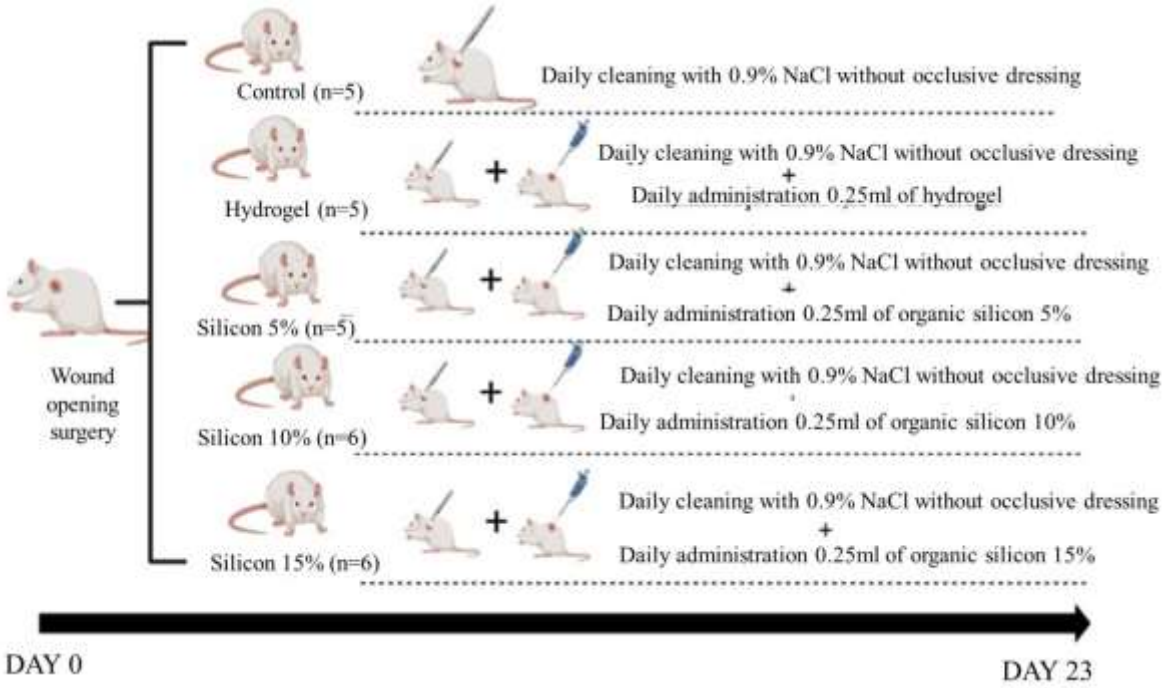


Figure 1 - Surgical template of a 2 cm² area before the procedure and the resulting surgical incision.

Stage 3

Animal treatment was performed daily following wound creation. The administered dose was standardized using a 100 IU insulin syringe with a volume of 25 IU (equivalent to 0.25 mL of product), containing concentrations of 5%, 10%, or 15% (Silicon 5%, 10%, and 15% groups, respectively), or the standardized commercial product hydrogel (Helianto, Hydrogel group), or wound cleansing with saline solution (0.9% NaCl, Control group). All experimental groups received daily wound cleansing with 0.9% NaCl solution without occlusive dressing.



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Other observations:

Please write like this: 5% organic silicon, 10% organic silicon, and 15% organic silicon.

Figure 2 - Experimental groups and their respective interventions. Created with BioRender.com.

Macroscopic Evaluation of Lesions

Lesions in animals from all groups were observed and recorded throughout the treatment period, focusing on wound characteristics, including the formation of the fibrin clot plaque. Data were tabulated according to the time required for complete detachment of the fibrin plaque for each animal, as well as the time to complete wound closure and re-epithelialization.

Photographic Documentation of Lesions

Photographs were used to evaluate fibrin clot detachment and wound healing, as well as for statistical analyses. Images were taken every two days, starting on day 1, immediately after surgery. The treatment period lasted 23 days, consistent with the study by Steffani et al.[16].

Euthanasia

At the end of the study, animals were euthanized by decapitation using a rodent guillotine, without anesthesia, to obtain whole blood for hematological analysis. The severity grade of this procedure is classified as "without recovery." Although euthanasia under anesthesia would have been preferable, it was incompatible with the objectives of this study, as anesthetic agents commonly used in animal experiments induce significant hyperglycemia in rodents [17].

Decapitation was performed with a guillotine in a facility exclusively designated for this purpose, with complete ventilation and sanitation of all equipment between animals. The procedure was carried out at the Biological Testing Laboratory under the supervision of a veterinarian (head of the UNIJUÍ Animal Facility) and a team with prior experience.

Biochemical and Hematological Analyses

Complete Blood Count (CBC)

To determine hematological parameters, blood was collected into tubes containing anticoagulant (EDTA) (5 µL of EDTA per 500 µL of blood). Automated determinations were performed using the Micros 60® hematology analyzer (Horiba), following the manufacturer's recommendations. The following parameters were obtained: red blood cell count (RBC); hematocrit (HCT); hemoglobin (HGB); erythrocyte indices, such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC); red cell distribution width (RDW); total leukocyte count; relative and absolute leukocyte count (neutrophils, eosinophils, basophils, lymphocytes, and monocytes); and platelet count. Samples were diluted 1:2 with 0.9% saline solution and analyzed in

triplicate. Subsequently, blood smears were prepared on slides, stained using the May–Grünwald Giemsa method, and analyzed by an experienced professional. For each slide, a count of 100 cells was performed.

Liver Enzymes

Alkaline phosphatase, alanine aminotransferase (ALT), and gamma-glutamyl transferase (γ-GT) levels were measured using kinetic methodologies with endpoint reactions in serum samples. For these analyses, Labtest® kits were used, and readings were performed with the Audmax 249 – Labtest automation system, using approximately 50 µL of serum per animal and 300 µL of reagent for each test performed. Results were expressed in U/L.

Renal Metabolites

Creatinine levels were measured using kinetic methodologies with endpoint reactions in serum samples. For these analyses, Labtest® kits were used, and readings were performed with the Audmax 240 – Labtest automation system, using approximately 50 µL of serum per animal and 300 µL of reagent for each test performed. Results were expressed in U/L. Uric acid levels were expressed in mg/dL.

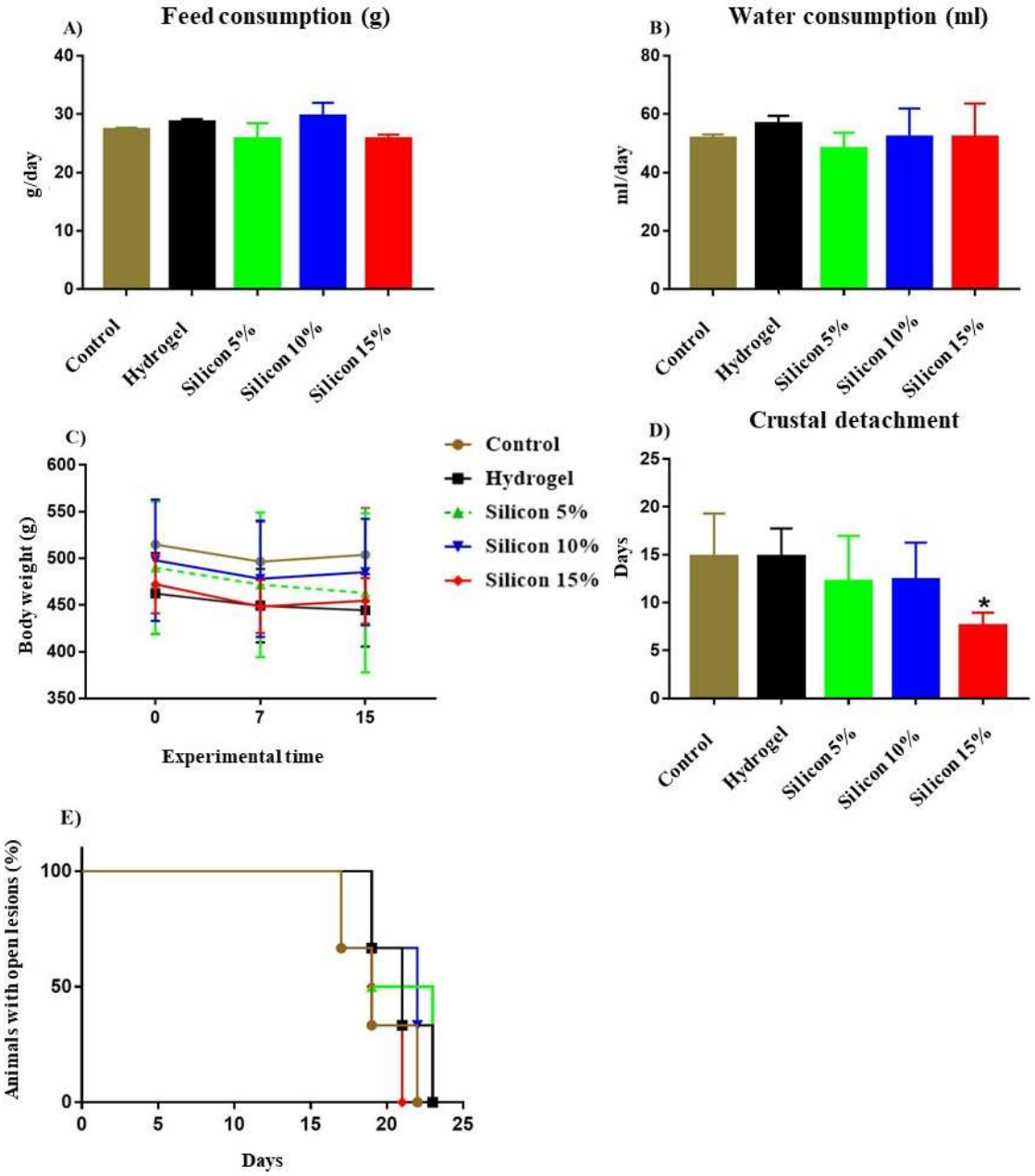
Statistical Analysis

Statistical analysis was performed using descriptive procedures (measures of central tendency and dispersion) and inferential tests (one-way and two-way ANOVA with repeated measures, followed by Tukey's post-test and Pearson's correlation test). All statistical analyses were carried out using GraphPad Prism 7.0, considering a significance level of $P<0.05$.

RESULTS

Regarding daily food and water intake, no differences were identified between the evaluated groups. We also did not observe differences in the body weight of the animals in the experimental groups throughout the study, demonstrating that silicon-based products did not alter the animals' feeding behaviors, similar to the control and hydrogel-treated groups (Figure 3A, B, and C).

We identified that treatment with 15% organic silicon accelerated the detachment of the fibrin clot in second-intention healing wounds, resulting in complete clot removal by day 10 of the experiment (Figure 3D). In addition, organic silicon at a 15% concentration showed greater speed in the complete closure of the wounds in all animals (Figure 3E), proving to be the concentration of choice for the treatment of this type of wound.



Please write like this: 5% Silicon, 10% Silicon, and 15% Silicon.

Figure 3 - Evaluation of food intake (A), water intake (B), and body weight during the experimental period. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn's post-test (A, B, and D), two-way ANOVA followed by Tukey's post-test (C), and descriptive analysis of the percentages of tissue repair and wound healing during the experimental period (E).

In the hematological and biochemical evaluations, no differences were found between the laboratory test results of the experimental groups. Thus, organic silicon did not demonstrate any toxic effects on hematological, metabolic, hepatic, or renal laboratory parameters.

Table 1. Analysis of laboratory parameters in the different experimental groups.

Laboratory Parameters	Control	Helianto Hydrogel	5% Silicon	10% Silicon	15% Silicon	P
RBC (10^6 /mm³)	6.9 ± 0.56	7.76 ± 0.29	7.10 ± 0.71	7.22 ± 0.43	7.14 ± 0.42	0.07
HGB (g/dL)	12.5 ± 0.99	13.7 ± 0.83	13.8 ± 1.83	13.2 ± 0.74	12.9 ± 0.70	0.42
HCT (%)	35.8 ± 2.9	39.9 ± 2.1	39.5 ± 4.3	37.9 ± 2.5	37.4 ± 2.5	0.24
MCV (L/fm³)	52 ± 1.41	51.2 ± 1.78	53.8 ± 1.30	52.2 ± 1.30	52.33± 1.50	0.14
MCH (L/pg)	18.1 ± 0.28	17.6 ± 0.70	19.4 ± 0.4	18.3 ± 0.54	18.1 ± 0.50	0.16
MCHC (g/dL)	35.0 ± 0.78	34.2 ± 0.43	34.8 ± 1.45	34.9 ± 0.59	34.6 ± 0.80	0.49
RDW (%)	13.5 ± 1.41	13.0 ± 0.25	13.7 ± 0.69	12.9 ± 0.48	13.0 ± 0.52	0.3
WBC (10^3/mm³)	2.44 ± 0.71	2.76 ± 0.47	2.82 ± 1.49	2.8 ± 0.50	3.46 ± 1.22	0.75
Neutrophils (10^3/mm³)	520 ± 153	558 ± 102	590 ± 270	610 ± 109	694 ± 228	0.74
Eosinophils (10^3/mm³)	24.4 ± 7.12	27.6 ± 4.77	28 ± 11	28 ± 5.09	34 ± 12	0.75
Basophils (10^3/mm³)	0	0	0	0	0	0
Monocytes (10^3/mm³)	19.2 ± 6.22	20.3 ± 3.22	21.4 ± 7.00	18.2±9.86	25.9 ± 9.07	0.85
Lynphocytes (10^3/mm³)	1,696 ± 492	1,970 ± 345	1,986 ± 799	1,942 ± 353	2,474 ± 907	0.73
PLT (10^3/mm³)	764 ± 121	866 ± 92	774 ± 106	736 ± 98	866 ± 77	0.11
PCT (%)	517 ± 72	618 ± 85	527 ± 73	482 ± 48	585 ± 67	0.07

MPV (fm^3)	6.8 ± 0.33	7.16 ± 0.35	8.82 ± 4.53	6.58 ± 0.23	6.75 ± 0.32	0.23
PDW (%)	9.6 ± 1.30	9.0 ± 2.09	10.5 ± 0.40	10.3 ± 1.33	9.0 ± 3.88	0.32
Alkalyne Phosphatase (U/L)	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	0
ALT (U/L)	74.0 ± 14.50	72.8 ± 16.76	84.4 ± 21.15	78.7 ± 14.48	94.8 ± 35.22	0.69
GGT (U/L)	7.0 ± 0.0	7.0 ± 0.0	7.2 ± 0.44	7 ± 0.0	7 ± 0.0	0.35
Uric Acid (mg/dL)	0.85 ± 0.60	0.30 ± 0.27	0.50 ± 0.0	0.50 ± 0.0	0.86 ± 0.63	0.9
Creatinine (mg/dL)	0.25 ± 0.04	0.28 ± 0.06	0.27 ± 0.02	0.28 ± 0.11	0.27 ± 0.02	0.82

Caption: Mean ± standard deviation of hematological, metabolic, hepatic, and renal parameters on the day of euthanasia. Statistical analysis was performed using the Kruskal-Wallis test and one-way ANOVA. RBC = Red blood cell count; HGB = Hemoglobin; HCT = Hematocrit; MCV = Mean corpuscular volume; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; RDW = Red cell distribution width; WBC = White blood cell count; PLT = Platelet count; PCT = Plateletcrit; MPV = Mean platelet volume; PDW = Platelet distribution width; ALT = Alanine aminotransferase; GGT = Gamma-glutamyl transferase.

DISCUSSION

Organic silicon at 15% proved to be an innovative product for accelerating the healing time of skin lesions in animals. This concentration of silicon accelerated both fibrin clot detachment and tissue proliferation, leading to complete wound closure after re-epithelialization, thereby demonstrating its potential use for the treatment for wound healing by second intention.

The fibrin clot is formed during the inflammatory phase of lesions, and its persistence in the wound bed (scab) is characterized as devitalized tissue[18]. Following the formation of this scab, the proliferative phase can be observed, which occurs through macrophage activation and fibroblast migration for the synthesis of collagen and elastin. These processes characterize the final stage of wound healing, as the regenerated tissue initiates the detachment of the fibrin plaque [19]. To support the healing process, different types of wound dressings aim to allow and enhance tissue proliferation, providing a curative and protective function for skin integrity as

quickly as possible, thereby preventing secondary complications such as infections or additional tissue lesions resulting from the contact of non-intact skin with different substances.

Hydrogel is a therapeutic agent that promotes hydration of lesion tissues and consequently maintains a moist environment, which favors wound healing. Moreover, this product contains calcium alginate, which facilitates the debridement of devitalized tissue, contributing to tissue proliferation. However, organic silicon, in addition to tissue hydration, improves skin characteristics such as elasticity, flexibility, and induction of new collagen fiber production[20].

Silicon is an essential component of fibrous structural proteins, glycoproteins, proteoglycans, and hyaluronic acid[20], which in turn contribute to tissue structure and repair [21]. Silanetriol (organic silicon) has already been demonstrated to be a trace element necessary for the formation of connective tissues (skeletal muscle), as well as for the synthesis and reorganization of collagen and elastin fibers, promoting regeneration, flexibility, and skin support. This highlights its therapeutic potential in the healing of second-intention wounds [9]. Previous studies have also shown that silicon may play an important role in cell proliferation by stimulating the synthesis of angiogenic growth factors, fibroblasts, and endothelial cells[22]. These factors are essential for tissue renewal and the formation of intact tissue to reestablish the chemical and biological barrier functions of the skin. Accordingly, the healing process with 15% silicon occurred more effectively compared to the other groups (21 days) (Figure 3D and E).

A reduction in silicon concentration in the body is associated with connective tissue disorganization and skin aging signs. Thus, previous studies have shown that topical organic silicon may attenuate such tissue disorganization and aging signs, which is related to tissue repair[23]. Oxidative stress, caused by an increased concentration of reactive oxygen species (ROS) relative to antioxidant defenses, can delay tissue healing and aggravate skin lesions[24]. However, silicon is capable of neutralizing ROS and mitigating their role in cellular damage[23], which may be related to the accelerated wound healing demonstrated in our study (Figure 3D).

Another relevant aspect to be considered in a pharmacological intervention is the evaluation of the potential toxicity of a new therapy[10]. In this regard, when evaluating the exposure of animals to different concentrations of silicon (5%, 10%, and 15%), no differences were observed in feeding behavior between the groups, either in food or water intake (Figure 3A and B), which consequently reflected in the absence of changes in body weight (Figure 3C). The fact that feeding behavior and body weight were not altered suggests that the therapeutic intervention in our study did not cause pain or a consequent reduction in appetite, as previously demonstrated[18].

The safety of the pharmacological intervention was also demonstrated through hematological analyses, which showed no differences among the surveyed groups, indicating that the interventions did not affect hematopoiesis. Likewise, analyses of hepatic and renal profiles revealed no differences in liver enzymes (alanine aminotransferase, gamma-GT, and alkaline phosphatase) or renal markers (uric acid and creatinine) (Table 1). This indicates the safety of using organic silicon for treatment in animals, similar to hydrogel [10].

In conclusion, 15% organic silicon accelerates the healing of second-intention wounds. Furthermore, organic silicon products did not exhibit hematological, metabolic, hepatic, or renal toxicity and did not alter the animals' feeding habits or body weight (Figure 4).

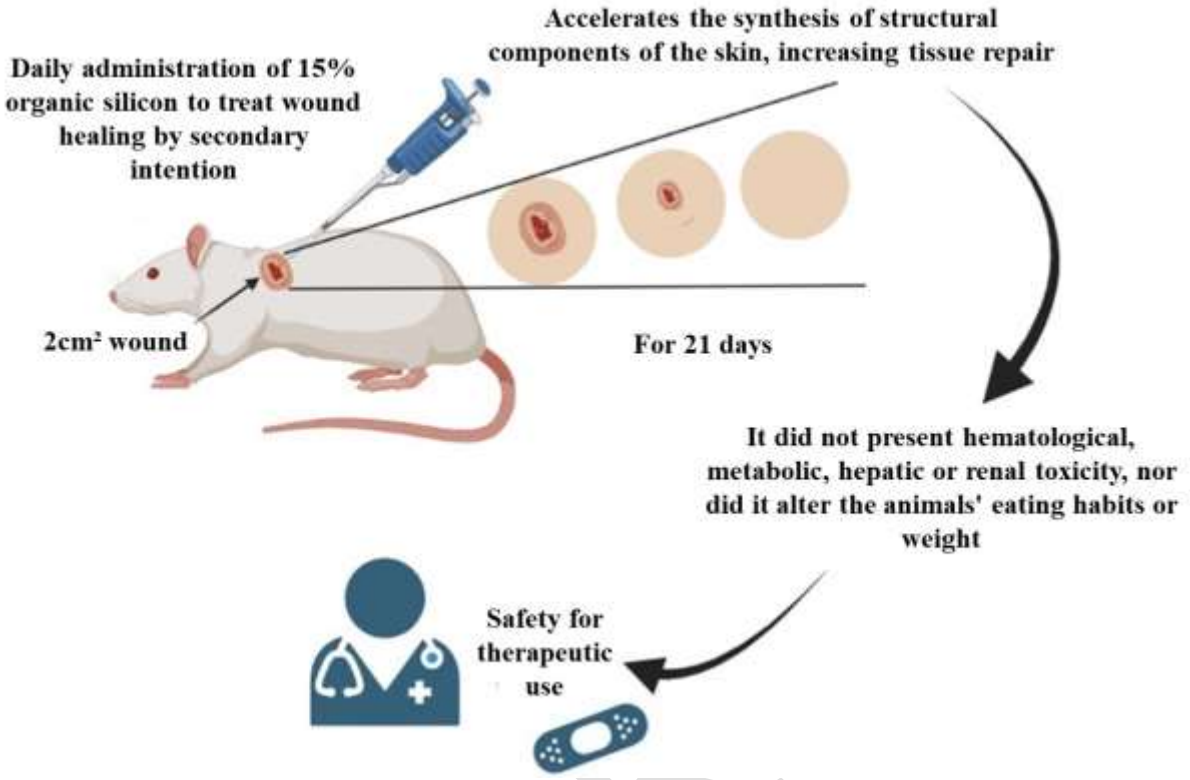


Figure 4 - Treatment with 15% organic silicon and tissue repair in wound healing by secondary intention. Created with BioRender.com.

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ORGANIC SILICON IN THE ACCELERATION OF TISSUE HEALING
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