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Effectiveness of Revolutic Biospray Preparations Against Wound Healing

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A R T I C L E I N F O

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A B S T R A C T

The skin has a major function in protecting the body from environmental influences. If it is injured, it will interfere with activities, cause pain, and facilitate infection. Furthermore, if this wound is wide and deep, it will be difficult to heal. Wound healing essential for the restoration of severed tissue, in this case, the skin, both anatomically and functionally. Damaged tissue will start the wound healing process, which is replacing damaged tissue or necrotic tissue with new and healthy tissue. Based on this explanation, the researcher interested in studying the role of Topical Biospray by Nutric on the number of PMN Leukocytes (neutrophils), Macrophages, Fibroblasts, Epithelialization, Cytokines Transforming Growth Factor – beta (TGF – β), and Wound Diameter in accelerating the wound healing process. This research was conducted at the Animal Laboratory of the Faculty of Medicine Hasanudin University, the Anatomy and Physiology Lab of Hasanuddin University, and the RSP Lab of Hasanudin University, applying the Randomized Post Test Control Group research design, using Wistar rats as research subject which were divided into 3 groups with different conditions and conditions. Revolutic Biospray applied topically to Wistar's wound. From the research conducted, it is proven that the relationship between wound healing using Biospray Revolutic compared to 0.9% NaCl solution and Biospray Plus preparations can provide very significant results at the stages of wound healing in the inflammatory, proliferative and maturation phases.

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INTRODUCTION

The skin has a major function in protecting the body from environmental influences. If it is injured, it will interfere with activities, cause pain, and facilitate infection. Furthermore, if this wound is wide and deep, it will be difficult to heal Wound healing essential for the restoration of severed tissue, in this case, the skin, both anatomically and functionally (Begum, 2000).

The damaged tissue will start the wound healing process, which is replacing damaged tissue or necrotic tissue with new and healthy tissue (Rodhiyah & Sulistiyawati, 2011). In the process of wound healing, phases that play an important role include the coagulation phase, inflammatory phase, proliferation phase, and maturation or remodeling phase (Atik Nur &; Iwan Januarsih, 2009; Hapsariani, 2014). The process of vasoconstriction, hemostasis, and also infiltration of inflammatory cells occurs in the inflammatory phase which begins within minutes after the wound and lasts up to several days (Puti *et al*, 2011). In this phase, there is also a vascular reaction at the site of injury characterized by the number of inflammatory cells bound to the wound and actively moving with lekosites such as polymorph nuclear leukocytes (PMN L) or neutrophils (Hapsariani, 2014). PMN cells are necessary, especially when an injury occurred, with an increase in the number of PMN cells as a response to movement from body cells. It is called chemotaxis produced by microorganisms (Hapsariani, 2014).

Signs of inflammation subside such as *rubor, calor, tumor, dolor*, and *function laesa* (Wijaya Y.A *et al*, 2015). Furthermore, the proliferative phase can be noticed by the presence of epithelialization, angiogenesis, and fibroblast proliferation starting on the third day after wounding and lasting for about 2 weeks afterward. This phase is the formation of granulation tissue in the wound itself, so *macrophages* and *lymphocytes* still play a role (Rodhiyah &; Sulistiyawati, 2007; Velnar*et al*, 2009). The stage of proliferation and epithelization is an important component used as a parameter to determine the success of wound healing. If on the wound there is no re-epithelialization, then the wound could not be considered as healed. Re-epithelialization is a stage of wound repair that includes mobilization, migration, mitosis, and differentiation of epithelial cells. These stages will restore the lost integrity of the skin. The onset of skin re-epithelialization will occur through the movement of epithelial cells from the edge of free tissue to damaged tissue. Wound healing is greatly influenced by re-epithelialization because the faster the re-epithelialization process. So, the faster the wound is closed, the faster the wound healing. The speed of wound healing can be influenced by the substances contained in the drug given, if the drug has the ability to increase healing by stimulating faster growth of new cells in the skin (Isrofah, 2013).

The final stage of the wound healing process, the remodeling phase, is characterized by tissue and collagen remodeling, epidermal maturation, and wound shrinking. In other words, this phase is very responsible for the development of new epithelium and the formation of final scar tissue. The remodeling phase itself can last 1 or 2 years, or sometimes for a longer period of time (Velnar *et al*, 2009).

Biospray by Nutric is an herb made from Colostrum cow's milk and soybean juice. It consists of 2 (two) types, namely Revolutic Biospray which contains L-Arginine, L-Ornithine, L-Glutamine, and L-Lysine, and Biospray Revolutic Plus containing Growth Factor (IGF-1, IGF-2, and IGF-β) Immune Factor (IgG, IgA, and IgM) Amino Acid (L-Glutamine, L-Lysine, L-Arginine, and L-Ornithine) Vitamin (vitamin A, vitamin B1, vitamin B2, vitamin B3, vitamin B6, vitamin B9, vitamin B12, vitamin C, vitamin D and vitamin E), and minerals (Calcium. Chromium, Iron, Magnesium, Sodium, Phosphorous, Selenium, Potassium, Zinc, Copper).

Biospray Revolutic as a product containing Arginine Ornithine, Lysin will work as a Reactive Oxygen Species (ROS) which is very strong in mitochondria and it also can accelerate the healing of various diseases. The content of Glutamine, Ornithine, and Arginine is ultimately also useful for increasing proliferation and improving the function of macrophage cells.

According to the research conducted by Daslina1, Eryati Darwin2, A.Aziz Djamal 2015), glutamine is an amino acid found in the body and one of its functions can modulate body immunity. It is seen that the percentage of phagocytosis against p. aeruginosa is smaller because of the ability of bacteria to deal with macrophages compared to latex

Furthermore, the research conducted by Ary Andini (2020) explaining that the amino acid glycine functioned in collagen synthesis which has an important role in connective tissue, glutamine plays a role during the inflammatory phase and the proliferation of wound healing as well as acts as an energy source, while arginine plays a role in immune function and stimulates endothelial cell function. The combination of these three amino acids is able to improve the healing of the patient's wound. The previous descriptions explained the important role of leukocyte PMN (neutrophils) in the inflammatory phase and epithelialization in the proliferative phase in the wound healing process.

★ Star Billionaires Club ★

PT Star Billionaires Klub is a legal entity that is a Biospray by Nutric Suplayer in Indonesia with a Vision of Prospering all Indonesian people and carrying out the mission of improving the health of the Indonesian people and improving the economy of the Indonesian people is the sponsor for this research.

Based on the explanation, the researcher is interested in examining the role of Revolutic Biospray topically on the number of PMN leukocytes (neutrophils), macrophages, fibroblasts, epithelialization and TGF – β, in accelerating the wound healing process and the difference in wound diameter

in the Biospray Revolutic group Nacl group 0.9% with the Biospray Revolutic Plus group.

THEORETICAL REVIEW

Wounds can be classified based on their anatomical structure, nature, healing process and duration of healing. As for the properties, namely: abrasion, contusion, incision, laceration, open, penetration, puncture, sepsis, etc. While the classification based on the structure of the skin layer includes: superficial, which involves the epidermal layer; partial thickness, which involves the layers of the epidermis and dermis; and full thickness involving the epidermis, dermis, fat layer, fascia and even to the bone (Kurniati, 2012).

Types of acute (new) injuries include surgical injuries, accident injuries, and burns. If the treatment is correct and the wound closes within 21 days, it is said to be an acute wound, otherwise it will fall into chronic wounds (wounds that are difficult to heal). Examples of acute injuries include surgical wounds that after less than 21 days have closed, or burns that heal during 21 days of treatment. Wounds are said to be chronic wounds, for example in accident injuries, new wounds will experience an inflammatory process for up to 5 days, if signs of inflammation are found on day 7 it may no longer be inflammatory but infection, and this can already be said with chronic wounds. It is said that chronic wounds due to prolonged inflammatory processes do not correspond to the physiological time of wound healing. Another example of chronic wounds in wounds with red wounds has been one month (> 21 days) does not want to close, so it can also be called a chronic wound (Arisanty, 2012).

The final stage of the wound healing process, the remodeling phase is characterized by tissue and collagen remodeling, epidermal maturation, and wound shrinking or in other words this phase is very responsible for the development of new epithelium and the formation of final scar tissue. The remodeling phase itself can last 1 or 2 years, or sometimes for a longer period of time (Velnar et al, 2009).

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Biospray by Nutric as a product containing Arginine Ornitihine, Lysin will work as a Reactive Oxygen Species (ROS) which is very strong in mitochondria can also accelerate the healing of various diseases. The content of Glutamine, Ornitihine, and Arginine in akhinta is also useful to increase proliferation and improve the function of macrophage cells. The proliferation of macrophages that occur in the bone marrow may increase because glutamine will be a precursor to arginine. Arginine may increase the proliferation and

function of macrophages in the bone marrow. This amino acid has an important role in the metabolism of macrophage cells involved in the process of innate immunity. These immunocompetent cells are capable of phagocytosing pathogens, killing fungi, and producing nitric oxide (NO), interleukins, tumor necrosis factor α (TNFα) and reactive oxygen species (ROS). Glutamine is able to stimulate macrophages to increase arginase secretion and provide Nicotinamide Adenine Dinucleotida Phosphate (NADP) thereby increasing NO secretion by macrophages. Nitric oxide plays an important role in the mechanism of bacterial killing.

Research conducted by Ary Andini, 2020 explained that the amino acid glycine plays a role in collagen synthesis which plays an important role in connective tissue, glutamine plays a role during the inflammatory phase and the proliferation of wound healing as well as acts as an energy source, while arginine plays a role in immune function and stimulates endothelial cell function. The combination of these three amino acids is able to improve the healing of the patient's wound.

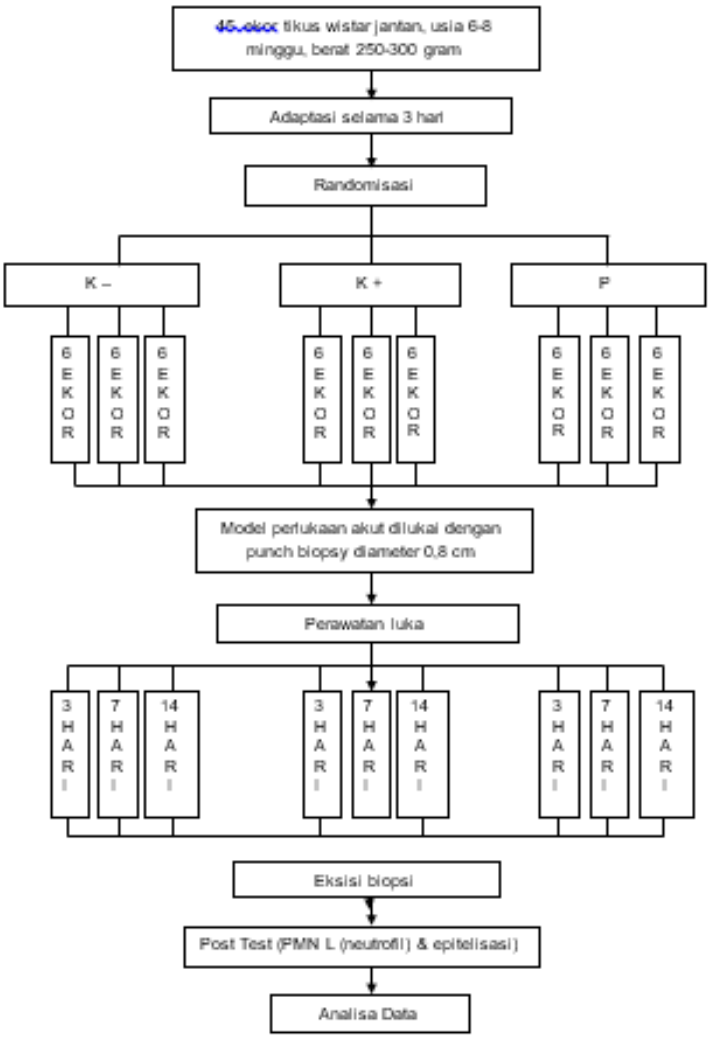
Based on this, researchers are interested in examining the role of Biospray by Nutric topically on the number of PMN leukocytes (neutrophils) and epithelialization, macrophages, fibroblasts, cytokines Growth Factor Transforming Growth Factor – β (TGF – β), and wound diameter in accelerating the wound healing process.

METHODOLOGY

This study used a *Randomized Post Test Control Group* research design using wistar rats as research subject. Wistar rats were divided into 3 groups, namely negative control, control, and Biospray Revolutic in which each group was divided into 3, namely day -3, day -7, and day -14:

The treatment given was being in the form of topical administration of Revolutic Biospray on acute wound model rats every day until day 14, with results in the form of the number of PMN L (neutrophils), macrophages, fibroblasts, epithelialization and TGF – β. After excision, then carried out wound treatment was with 0.9% Nacl Solution, Biospray Revolutic, Biospray Revolutic Plus.

The research procedure carried out was making acute injury models and wound care. Rats were dissected with existing procedures so that circular wounds were obtained (Rodhiyah, 2011).



Wound treatment on wistar was carried out daily by spraying Biospray Revolitic and Biospray Revolutic Plus and 0.9% Nacl Solution on wounds in each group of rats. Then the data collection procedure is carried out after obtaining research permission and *Ethical Clearance*, *biopsy* excision procedure then the procedure for making histopathological preparations with stages of organ fixation, washing and dehydration, embedding / soaking, cutting, cutting tissue blocks, staining with the HE (*Hematoxylin - eosin*) method, and finally PMN L (neutrophil) histopathology examination and epithelialization.

After the data is submitted, it is analyzed using the *SPSS* computer program with a 95% confidence degree and a $\alpha \leq$ value of 0.05. Univariate analysis was carried out on each variable studied to determine the picture of frequency distribution and data normality of all research variables. The data collected was then tested for homogeneity using the *Levene* test and data normality using the *Shapiro Wilk test*. Bivariate analysis is performed to determine the relationship between variables, if the distribution of normal data is carried out *Independent Sample T Test*, 81 while if the distribution of data is abnormal using the Mann Whitney test.

RESULTS

In this study, the number of samples was 45 samples consisting of 3 groups, each group consisting of 15 male wistars with a reserve of 2 heads per group. Acute modeling was carried out by excision of the upper back with a diameter of 8 mm using PUNC Byopsies and for each stage of time 3 days, 7 days and 14 days sacrifice was executed.

Treatment was carried out on experimental animals in the control group wistar wounds were treated by applying 0.9% NaCl to excision wounds on the back, Treatment I wistar wounds were treated by spraying Biospray Revolutic preparations on wistar back excision wounds, Treatment II group, wistar wounds were treated by spraying Biospray Revolutic Plus on wistar back excision wounds.

Furthermore, the results of the study were analyzed using the SPSS program and described as follows:

Univariate Analysis

1. The effect of topical administration of Revolutic Biospray on the amount of PMN L (neutrophils) in the wound healing process in wistar rats with acute injury models. From the results of this experiment, it can be seen that revolutic biospray has a high effect on reducing the amount of PMN-L on the 14th day of treatment when compared to the administration of revolutic plus biospray and NaCl administration.
2. The effect of topical administration of Biospray Revolutic on the number of macrophages in the wound healing process in wistar rats with acute injury models. From the results of this experiment, it can be seen that revolutic biospray has a high effect on reducing the number of macrophages on the 14th day of treatment when compared to the administration of revolutic plus biospray and NaCl administration.
3. The effect of topical administration of Biospray Revolutic against fibroblasts in the wound healing process in wistar rats with acute injury models. From the results of this experiment, it can be seen that on the 14th day topical administration of Biospray Revolutic is significant and is considered more effective because it has a high effect on fibroblast formation in the wound healing process in wistar rats with acute injury models when compared to NaCl and biospray revolutic plus.
4. The effect of topical administration of Biospray Revolutic on epithelialization in the wound healing process in wistar rats with acute injury model. From the results of this experiment, it can be seen that on the 7th day topical administration of Biospray Revolutic is significant and effective because it has a high effect on epithelialization in the wound healing process in wistar rats with acute injury models when compared to NaCl and biospray revolutic plus.
5. Differences in cytokine expression Transforming Growth Factor – β (TGF – β) in the Biospray Transform group, Biospray Plus Group with NaCl group 0.9% From the results of this experiment, it can be seen that the revolutic biospray given for 3 days proved effective and had a major

impact on increasing the expression of cytokine Transforming Growth Factor – β (TGF – β) compared to the administration of biospray revolutic plus and NaCl administration of 0.9 % on day 3 and decreased significant on day 7 and day 14.

6. The difference in wound diameter in the Biospray Revolutic group of the Biospray Plus group with the Nacl group is 0.9% From the results of this experiment, it can be seen that the revolutic biospray given since day 3 has had an effect on changing wound size and wound size getting smaller on day 7 and day 14 after administration of biospray revolutic compared to the wound size given Nacl 0.9% and biospray revolutic plus. Giving revolutic biospray is considered best for reducing wound size with an average reduction in wound size that is greater than other treatments.

A. Expression of the number of PMN L (neutrophils) to determine the effect of giving black soybean flour extract on leukocytes of rats implantation after ovariectomy. This study used a Complete Randomized Design (RAL) of 4 treatments and 7 repetitions each. Treatment 1 (negative control), treatment 2 (positive control: estradiol concentration 50 ppm), treatment 3 and 4 respectively with the administration of black soybean flour extract doses of 0.31 g / ml and 0.63 g / ml. The data was analyzed using the One Way Anova test with a confidence level of 95% or α 0.05 and further Duncan Multiple Range Test (DMRT) was carried out.

The results showed that administration of black soybean flour extract for 10 days in rats post-ovariectomy implantation showed a significant effect, namely doses of 0.31 g / ml and 0.63 g / ml were able to increase the total number of leukocytes and the number of agranulocyte leukocytes.

B. Macrophage expression to determine the effect of black soybean tempeh consumption on macrophage activity and IL-1 level in rats in vivo. A total of 30 rats were grouped into 5 (five), each group of 6 rats. For 30 days each group was maintained with standard diit and diit plus black soybean tempeh flour (25, 50, 75 and 100% instead of casein). After that, peritoneal fluid is taken to be used for analysis of macrophage activity and IL1 levels. The results showed that the higher the amount of black soybean tempeh in the feed, the higher the phagocytosis index and L-1 levels. Consumption of black soybean tempeh affects macrophage activity and IL-1 ($p<0.05$) levels. Increased macrophage activity is positively correlated with the amount of IL-1, with a correlation coefficient of 0.9.

C. Fibroblast expression can be concluded that on the 14th day topical administration of Biospray Revolutic is significant and considered more effective because it has a high effect on fibroblast formation in the wound healing process in wistar rats with acute injury models when compared to NaCl and biospray revolutic plus.

In line with the research of Arifah Nur Hasanah (2019), Genistein contained in soybean edamame seeds has anti-inflammatory, antibacterial and antioxidant properties. Edamame also contains vitamins A, C, and E which also have antioxidant properties. This study aimed to determine the effectiveness of ethanol extract of edamame seeds (ED) in increasing the number of fibroblasts in healing grade II burns. This research is a true experimental research with post test only control group design. The study sample was 24 grade II burn model rats made by attaching hot metal (60°C) for 5 seconds to the backs of shaved rats. The samples were divided into 6 groups with topical treatments, including positive control group (silver sulfadiazine), negative control group (Na CMC 0.5%), group with topical administration of edamame seed ethanol extract, consisting ED 20%, ED 40%, ED 60%, ED 80%. The treatment is carried out for 15 days and skin sampling is carried out on the 16th day. The preparation is made by staining Hematoxylin and Eosin. In one way ANOVA test, a significance value of 0.011 ($p \leq 0.05$) was obtained, which means that a significant difference in the number of fibroblasts between the 2 groups contained.

D. The expression of epithelialization is that on the 14th day topical administration of Biospray Revolutic is significant and effective because it has a high effect on epithelialization in the wound healing process in wistar rats with acute injury models when compared to NaCl and biospray revolutic plus administration.

This study is also in line with Annisa Nurul Aini's (2022) research that determining the effect of soybean edamame can increase epithelial thickness in burns, edamame contains several active ingredients that can accelerate the wound healing process, including isoflavones, vitamins A, C, and E. Isoflavones have the effect of accelerating wound healing by accelerating the rate of epithelialization through the induction of transforming growth factor One of the parameters of healing burns is to measure the thickness of the epithelium that is formed.

E. Differences in Cytokine Expression Transforming Growth Factor – β (TGF – β).

There is an increase in TGF-β levels at the end of the proliferation phase or the beginning of the remodeling phase. This leads to an increased proliferation of fibroblasts to synthesize collagen which can later become hypertrophic scars and keloids.

Related to the research conducted by Gusti Revilla (2019), papain is an enzyme found in papaya sap. This enzyme has catalytic activity that can reduce scarring due to burns. The purpose of this study was to determine the effectiveness of papaya sap papain on the levels of growth factor Transforming growth factor -β (TGF-β) in the healing process of rat burns. The study was experimental using 15 rats as research objects and divided into 3 groups, namely the control group, the group was given papain and the comparison group was given silver sulfadiazine. Rats were made partial burns by heating

metal in boiling water and attaching it to the dorsal part of the rat for 20 seconds. Rats suffering from burns were treated according to the group and on day 5 blood was taken through the eyes to obtain serum, then TGF-β levels were measured using the Elisa method. The results showed that the average level of TGF-β in the control group was 317.72 pg / ml, the group was given 186.24 pg / ml and the comparison group was 192.11 pg / ml. This suggests that the papain enzyme is able to reduce TGF-β levels which are proinflammatory so that papain may be able to accelerate the inflammatory phase and also be able to accelerate the wound healing process.

F. Expression The difference in wound diameter was obtained that the revolutic biospray given since day 3 had an effect on changing the wound size and the wound size was getting smaller on day 7 and day 14 after applying biospray revolutic compared to the wound size given NaCl and biospray revolutic plus. Research Vera Eufrasia (2019) Ethanol extract of red jeringau rhizomes contains flavonoids and saponins which are believed to help in accelerating the wound healing process. This study aims to determine the effectiveness of ethanol extract of red jeringau rhizomes on the wound healing process and determine the effect of different concentrations of extracts in the preparation on the wound healing process.

Experimental animals were divided into 5 groups, namely the negative group that was not given treatment, the positive group given povidone iodine ointment, the F5 group (extract concentration 5%), the F10 group (extract concentration 10%), the F15 group (extract concentration 15%) which was given an extract that had been made topically ointment. Observations were made with the Macbiophotonic Image J program by measuring the length of the wound from day 1 to day 9. Data analysis using One Way Anova and Post Hoc Test. Result.

Statistical analysis shown a significant difference with the research conducted by Teguh Sutrisno (2016). Burns are tissue damage caused by contact with heat sources and quercetin is thought to accelerate the healing of burns because it has anti-inflammatory, antibacterial, and antioxidant effects. The purpose of this study was to evaluate the effect of quercetin in accelerating the healing of grade IIA burns. The study used 45 white rats of Wistar strains which were grouped into 3, namely the treatment group on days 5, 11, and 21.

The burn is made with round metal 2 cm in diameter and 1 mm thick heated at 100°C for 10 seconds. Data were analyzed using the Kruskal-Wallis test and LSD with a confidence level of 95%. The results of the analysis showed that quercetin gel can accelerate the reduction of wound diameter on day 11 and reduce color intensity on day 21. The formation of collagen and sebaceous glands in quercetin differed significantly from the negative control pad.

CONCLUSIONS

a. Wound healing using Biospray Revolutic topically was better than the 0.9% Nacl group and Biospray Revolutic Plus with evidence in the final phase of healing showing PMN L (Neutrophil) cells were greatly decreased.
b. Wound healing using topical Revolutic Biospray was better than the 0.9% Nacl group and Revolutic Biospray with evidence in the final phase of healing showing a greatly decreased macrophage cell count
c. Wound healing using Biospray Revolutic topically is better than the 0.9% Nacl group and Biospray Revolutic with evidence in the final phase of healing showing a high effect on increasing fibroblast cell formation
d. Wound healing using Biospray Revolutic topically is better than the 0.9% Nacl group and Biospray Revolutic with evidence in the final phase of healing showing a high effect on increasing epithelial tissue formation
e. Wound healing using Biospray Revolutic topically was better than the control group with evidence in the final phase of healing showing decreased expression effect of cytokine Transforming Growth Factor – β (TGF – β) compared to the NaCl group 0.9% and Biospray Revolutic on day 7 and day 14
f. Wound healing using topical Revolutic Biospray was better than the 0.9% Nacl group and Revolutic Biospray with evidence in the final phase of healing showing the smallest wound diameter compared to the 0.9% Nacl group and Revolutic Biospray

RECOMMENDATION

This study is an evidence that explains the relationship between wound healing using Biospray Revolutic preparation compared to 0.9% Nacl solution and Biospray Plus preparation which can provide meaningful results at the stage of wound healing in the inflammatory, proliferation and maturation phases. Further studies are needed to be able to determine the mechanism of action of Biospray Revolutic preparations using other biomarkers. Furthermore, studies are needed on clinical application in humans because Biospray Revolutic preparations can heal wounds acutely.

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