

Impact of Local Insulin Injection on Split Thickness Skin Graft Donor Site Healing

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ABSTRACT

Background: Split thickness skin graft (STSG) remains the most common surgical technique to cover skin defects. However, the healing of its donor area is of paramount importance. Multiple local drugs are frequently used to enhance its epithelialization. Systemic Insulin was proved to be beneficial for chronic wound healing such as pressure sores and diabetic ulcers. But the impact of local insulin injection was scarcely studied before as regard wound healing.

Objectives: This study aimed to evaluate the effect of intradermal insulin injection on the donor of harvested split thickness skin graft (STSG).

Patients and Methods: In this study, 40 patients; 18 males and 22 females with skin loss planned to be covered by STSG were selected. Pregnant, lactating, pre-diabetics and diabetic patients were excluded. The dimensions of the raw area ranged from 5-10cm for maximum height and width. The thigh was used as a donor area and divided by a horizontal line into test (upper proximal half) and control (lower distal half) areas. Then, a 10 IU of long-acting insulin (Lantus®) was intradermally injected in the test area. Random blood sugar was measured 6 hours postoperatively. The rate of epithelialization on days 14 and 21 was analyzed by a special software (Image J®) and the scar quality was also evaluated by Vancouver scar scale (VSS).

Results: For the test area, the rate of epithelialization was significantly faster on days 14 and 21 as compared with the control area. But there was no statistical difference between both areas regarding the scar quality when using Vancouver scar scale.

Conclusion: Intradermal injection of insulin in donor sites of split thickness skin graft accelerates its healing which allows early re-harvesting of grafts from same donor sites.

Key Words: Insulin- Growth factors – Graft donor – Wound Healing.

Ethical Committee Approval: This work was conducted at Plastic, Burn and Maxillofacial surgery department, Ain Shams University. A written informed consent was obtained from all patients. The study was approved by the Research Ethical Committee of faculty of medicine under No (FWA

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INTRODUCTION

The split thickness skin graft (STSG) remains the golden standard to manage most of skin defects which occur secondary to trauma, tumors, burns, or even after surgical excisions [1].

Hence, the rapid and complete healing of its donor areas is of highest priority allowing multiple sessions of grafting from same donor site as in major burns. The main healing mechanism is the re-epithelialization from the remaining dermal appendages and hair follicles. This is completed mainly within 2-3 weeks depending on many factors such as patient's general condition and local conditions; donor wound location, depth, and bacterial contamination [2].

Multiple local drugs were used to enhance donor area healing such as platelet-rich plasma (PRP) [3] and heparin [4]. On the other hand, systemic application of insulin- as an anabolic hormone- has a positive effect on all phases of wounds healing. It reduces the reactive oxygen species (ROS), M2 macrophages and IL-10 levels, thus, decreasing their injurious effects on DNA, proteins, and lipids during the inflammatory phase [5].

Furthermore, Insulin and its derivatives as insulin like growth factor-1 stimulate proliferation of the keratinocytes at the donor site, and increase the production of transforming growth factor beta (TGF-β), which in turn accelerates the migration of the epithelial cells to the injected area of split thickness skin graft donor site [6]. Moreover, this effect is mediated through the PI3K-Akt-Rac1

pathway throughout insulin dependent receptors, insulin enforces the extracellular matrix and collagen synthesis by increasing the proliferation, growth and maturation of myofibroblasts with neovascularization during the proliferative phase [7].

During the maturation phase, it activates mitogen protein kinase signal transduction, enforces extracellular matrix, and stimulates development of fibroblasts and collagen remodeling [8].

Despite these advantages, systemic insulin has crucial disadvantages. It induces systemic hypoglycemia, hypoaminoacemia and hypokalemia [5]. So, local insulin injection was safely tried experimentally on rabbits [9] and on limited number of humans [10].

PATIENTS AND METHODS

A total number of 40 patients: 22 (55.0%) females and 18 (45.0%) males were included. Their ages ranged from 18 and 40 years with mean of 31.08 years. Diabetics, prediabetics, pregnant and lactating females were excluded. The local wound was assessed regarding the etiology, dimensions, site and depth. Wounds that were eligible for coverage by split thickness skin graft are included and wounds that necessitate dressing only, flap or heavily infected were excluded. All included wounds were photographed and documented.

Different etiologies of the acute wounds were included with 33 patients (82.5%) secondary to minor burn not exceeding 5% of total body surface area to avoid the systemic and local burn effects, 4 patients (10.0%) were post traumatic and 3 (7.5%) of wounds were due to surgical excisions.

Intraoperatively, the wound was re-assessed, debrided and measured. The anterior aspect of the thigh was sterilized and draped to be used as a donor for the skin graft. The graft size was marked to be 1.5-2 times the surface area of the raw area to be covered. Before graft harvest, a horizontal line was drawn to bisect the donor into 2 equal halves, an upper half which is considered the test area and a lower half for the control area. An electrical dermatome (Integra® Padgett®) was used for graft harvest with thickness adjustment to 0.010-0.015 inch and the test area was injected intradermal in 10 random points by 10 IU with a concentration of 1 IU/ml of long-acting insulin (Lantus®). This insulin dose was calculated to be the safest dose that could be tolerated without the need of exogenous glucose to correct hypoglycemia [8,11] (Fig. 1).

Then, the donor was dressed using a non-adherent layer-Vaseline gauze (Bio-Tulle®)-, multiple layers of dry sterile gauze, an absorptive wound pads and crepe bandage. The skin graft was then applied to the recipient area with tie over dressing (Fig. 1).

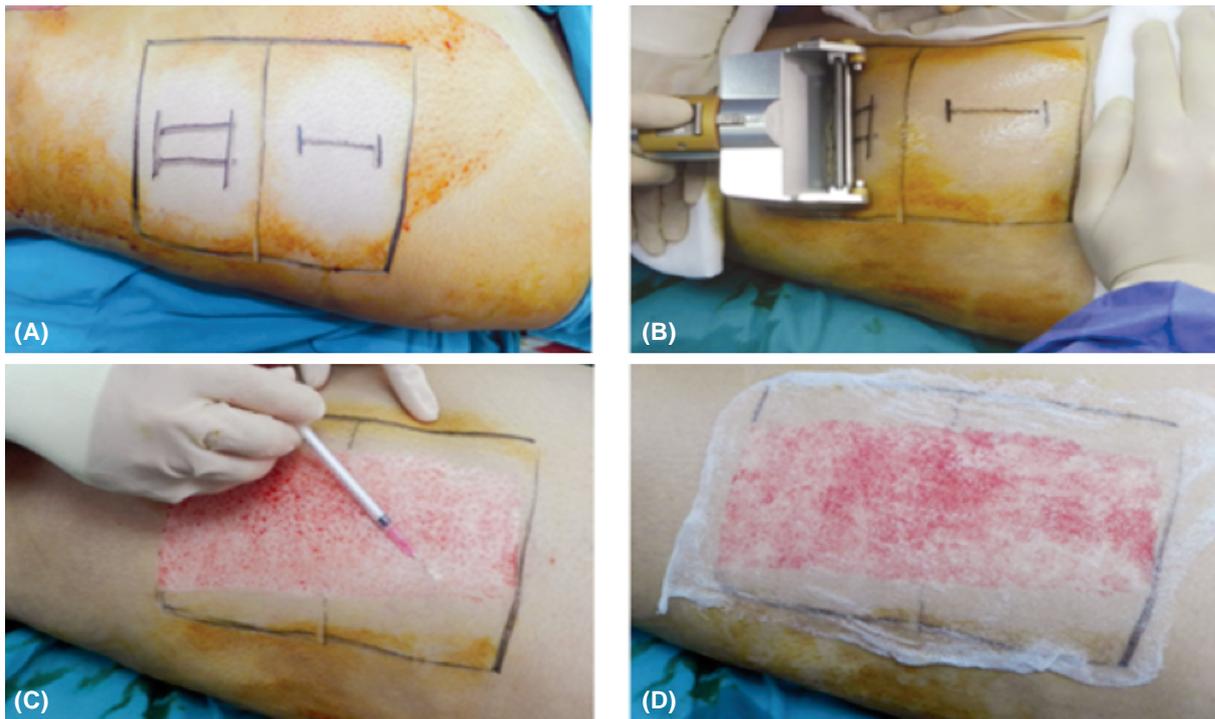


Fig. (1): 36-year-old female with post burn skin loss over right lower limb. Donor site was divided into 2 equal halves via a horizontal line; Test area (I) and Control area (II) (A); A medium thickness STSG was harvested using an (Integra® Padgett®) electrical dermatome (B); The test area was injected by 10 IU of long-acting insulin (Lantus®) (C); The donor is covered by Vaseline gauze-based material (Bio-Tulle®) (D).

Six hours post insulin injection; the random blood sugar was measured and documented. Donor wound dressing was checked at the first day post-operatively and after 3 days and if soaked, the absorptive pads were changed immediately. The first graft dressing was done on the 3rd postoperative day in post-traumatic wounds and on the 5th day in post-surgical wounds.

Patients' assessment: All donor areas were followed up clinically till complete wound healing. At days 14 and 21, all dressing layers over the graft donor were meticulously removed and wound was assessed clinically for signs of healing by two plastic surgeons independently and photographed. Another 10 IU of long-acting insulin (Lantus®) was injected at the non-healed islands of test area.

After that, for objective assessment, the rate of epithelialization and the non-healed/healed ratio were calculated for both test and control areas by a special Software (Image J®) using plot-profile. A two-dimensional graph of the intensities of pixels along a line is drawn for each control and test areas. In this graph, the X-axis represents the distance along the line while the Y-axis is the pixel intensity. The highest point in this graph represents the lightest intensity (= Area healed by epithelialization) while the lowest point represents the darkest area (= Area not healed). (Fig. 2).

The resultant scar quality was assessed subjectively using Vancouver scar scale which addresses the scar pigmentation, pliability, height and vascularity.

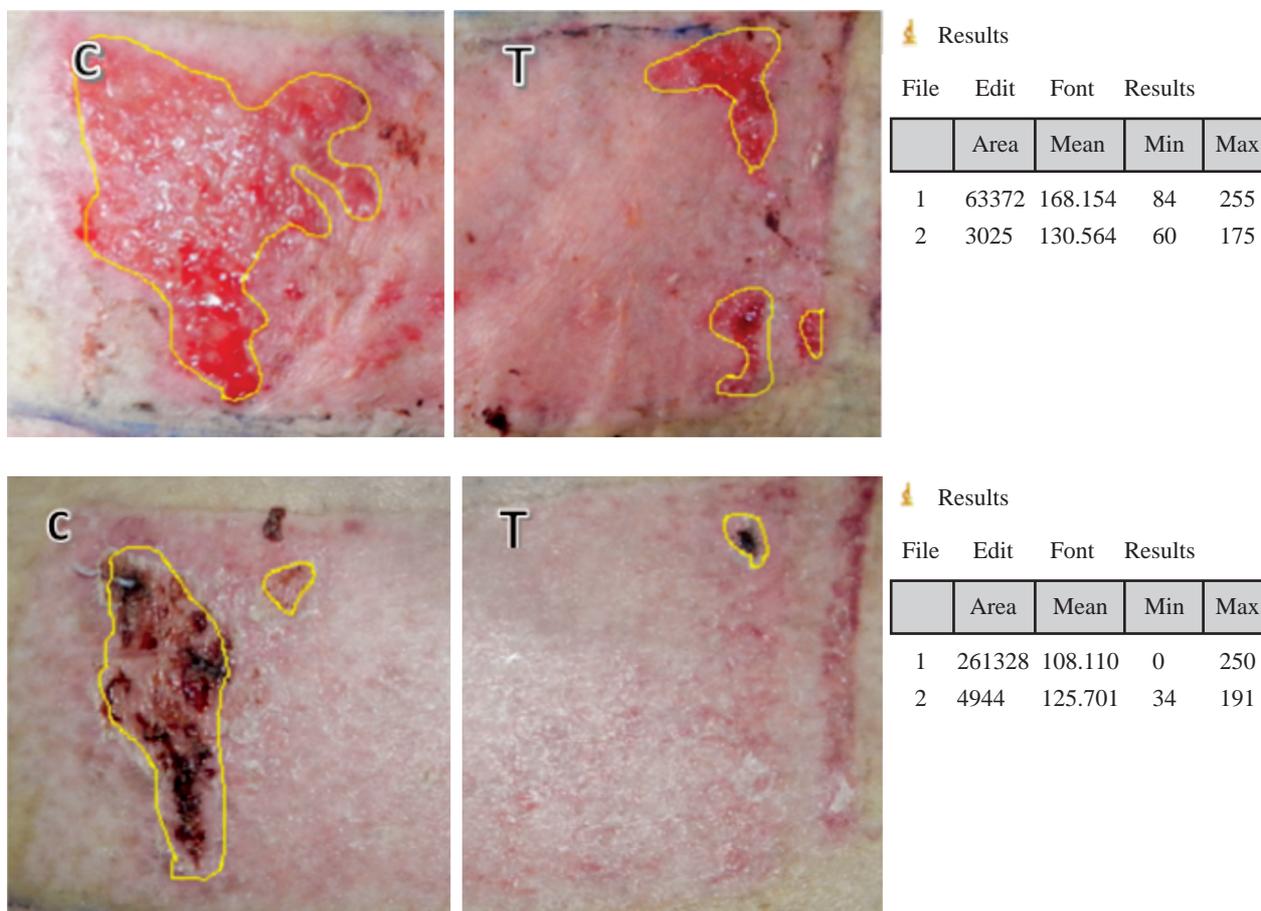


Fig. (2): Software analysis of the non-healed/Total wound area ratio using (Image J®) on days 14 and 21 post-operative for Test area (T) and control area (C), the non-healed areas are marked by yellow and surface areas were measured.

RESULTS

This study was conducted on 40 patients with 22 females (55.0%) and 18 males (45.0%) admitted to Plastic, Maxillofacial and Burn Department, Ain Shams University. The patients age ranged from 18-40 years with mean of 31.08 years. Statistically,

there was no significant correlation found between age of the patients and the rate of epithelialization.

Preoperative Random blood sugar level was of average $125 \pm$ SD of 26.58 and postoperatively was of average $130.28 \pm$ SD of 18.2. None of the patients experienced hypoglycemia.

Regarding rate of epithelialization using (Image J®) software, on day 14 postoperative, test area was healed by 73.44%±0.129 of the total wound area in contrary to the control area that healed by 57.85%±0.155 of the total wound area. On day 21 postoperative, the healing of the test area was increased to be 78.68%±0.0732 of the total wound area compared to control area healing which increased also to be 60.61%±0.153 of the total wound

area. Statistically, Rate of epithelialization was significantly increased at both days 14 and 21 in the test area than the control one with *p*-value = 0.010 and <0.001; respectively (Table 1).

Using Vancouver scar scale to assess the quality of scar, there was no significant difference found between both test and control areas with *p*-value = 0.074 (Table 2).

Table (1): Comparison between control area and test area regarding rate of epithelialization at day 14 and day 21.

Epithelialization	Control area No.= 40	Cases area No.= 40	Test value	<i>p</i> -value	Significance
<i>Day 14:</i>					
Mean ± SD	124.63±32.63	146.30±32.27	-2.672°	0.010	Significant
Range	73.6-176.44	86.71-195.31			
<i>Day 21:</i>					
Mean ± SD	125.18±28.74	175.40±17.77	-8.408°	0.000	Highly Significant
Range	82.91-188.72	113.54-197.57			
<i>Paired t-test:</i>					
Test value	0.094	5.673			
<i>p</i> -value	0.925 (NS)	0.000 (HS)			
<i>Rate of change:</i>					
Median (IQR)	15.23 (-13.65-21.31)	16.47 (8.38-44.18)	-2.162≠	0.031	Significant
Range	-77.66-45.32	-0.8-108.25			

p-value >0.05: Non significant. *p*-value <0.05: Significant. *p*-value <0.01: Highly significant.
°: Independent *t*-test. ≠: Mann-Whitney test.

Table (2): Vancouver scar scale for both donor and control areas.

Scar characteristic	Insulin Area Score (n=40)		Control Area Score (n=40)		<i>p</i> -value
	Median (IQR)	Min-Max	Median (IQR)	Min-Max	
Vascularity (0-3)	1 (1)	0-3	1.5 (1)	0-3	0.014
Pigmentation (0-2)	1 (0.5)	0-2	1 (0.75)	0-2	0.021
Pliability (0-5)	1 (1)	0-2	1.5 (1)	0-3	0.071
Height (0-3)	1 (0.5)	0-2	1 (0.5)	0-2	0.053
Total (0-13)	4 (2)	0-7	5 (2)	2-9	0.074

IQR = Inter-quartile range. Min = Minimum. Max = Maximum.
p-value >0.05: Non significant. *p*-value <0.05: Significant. *p*-value <0.01: Highly significant.

DISCUSSION

Split thickness skin grafts remain the simplest yet the most important surgical technique used to manage large skin defects. Post-operative, patients reported pain and discomfort at their donor site more than the graft-treated area. In addition, donor site healing may be associated with erythema, scarring, and loss of skin elasticity which delays the option of re-harvesting grafts from the same site. Various preventive and therapeutic measures; dressing materials, pressure garments, silicone gel

sheets, sprays had been used to reduce such morbidity [2].

Multiple studies have shown that systemic application insulin plays an important role in wound healing. With its ability to restore damaged skin. and its relatively affordable cost, experimenting its incorporation in wound dressings was steered [10].

These biological effects are associated with the phosphatidylinositol 3-kinase (PI3K) and mi-

togen-activated protein kinase signaling [12]. Via insulin-like growth factor-1 (IGF-1), it increases keratinocytes migration and differentiation [13]. Moreover, it boosts collagen deposition and maturation by increasing the levels of hydroxyproline, and endothelial cells proliferation [14].

However, application of systemic insulin for wound healing has a major drawback which is inducing systemic hypoglycemia, associated with hypokalemia and hypoaminoacidemia [15]. Due to these complications, topical insulin has been experimented to fasten wound healing with minimal systemic side effects [16].

At the Shriners Hospital, Texas, a study was conducted on pediatric major burn patients; continuous infusion of recombinant human insulin titrated to glucose level was applied. Results showed marked increase in the fractional synthesis rate of wound proteins in the early post-operative period [17].

Martínez-Jiménez et al., [18], have proven by sectional biopsies that, Insulin-treated donor areas showed significant increase in the number of new blood vessels with marked endothelial cells proliferation.

This study was concerned with the effect of intradermal insulin injection over STSG donor sites in a reliable number of non-diabetic patients. The current study demonstrated that intradermal injection of insulin in donor sites of STSG accelerates its healing, with no reported complications related to the use of insulin either by clinical or laboratory findings.

In the present study, the Vancouver Scar Scale was helpful to show that there's no significant changes between the conventionally treated areas and the insulin treated areas, regarding each of the five variables: Vascularity, pigmentation, pliability, thickness and height.

Limitations: Insulin effect couldn't be assessed regarding postoperative rates of infection, pruritus symptoms, and pain, as the test and the control areas were confined to the same site in each patient. Further studies are required with the sample size calculated for each of these outcomes.

Conclusion:

Intradermal injection of insulin in donor sites could have a role in its healing, with faster rate of epithelization which allows early re-harvesting of grafts from same donor sites. However, it does not

reduce post-operative pain nor improve the long-term scarring.

Ethical committee approval: This work was conducted at Plastic, Burn and Maxillofacial Surgery Department, Ain Shams University. A written informed consent was obtained from all patients. The study was approved by the Research Ethical Committee of Faculty of Medicine under No (FWA 000017585). All surgical steps were done by the most senior surgeon.

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REFERENCES

- 1- Kanapathy M., Hachach-Haram N., Bystrzonowski N., Connolly J.T., O'Toole E.A., Becker D.L., Mosahebi A. and Richards T.: Epidermal grafting for wound healing: A review on the harvesting systems, the ultrastructure of the graft and the mechanism of wound healing. *International Wound Journal*, 14 (1): 16-23, 2017.
- 2- Kumar P., Ajai K.S. and Sharma R.K.: The role of recruited minced skin grafting in improving the quality of healing at the donor site of split-thickness skin graft-A comparative study. *Burns*, 45 (4): 923-928, 2019.
- 3- Xu F., Zou D., Dai T., Xu H., An R., Liu Y., et al.: Effects of incorporation of granule-lyophilized platelet-rich fibrin into polyvinyl alcohol hydrogel on wound healing, 8 (1): 1-10, 2018.
- 4- Manzoor S., Khan F.A., Muhammad S., Qayyum R., Muhammad I., Nazir U., et al.: Comparative study of conventional and topical heparin treatment in second degree burn patients for burn analgesia and wound healing. *Burns*, 45 (2): pp. 379-386, 2019.
- 5- Dhall S., Silva J.P., Liu Y., Hrynyk M., Garcia M., Chan A., Lyubovitsky J., Neufeld R.J. and Martins-Green M.: Release of insulin from PLGA-alginate dressing stimulates regenerative healing of burn wounds in rats. *Clinical Science*, 129 (12): 1115-1129, 2015.
- 6- Leonov Y.I., Shkumat M.S., Klymenko P.P., Hovorun M. Y., Guzyk M.M., Kuchmerovska T.M. and Pishel I.M.: Effect of insulin-like growth factor transgene on wound healing in mice with streptozotocin-induced diabetes. *Cytology and Genetics*, 49 (1): 19-26, 2015.
- 7- Fang L., Wu H., Li X., Fang J. and Zhu Y.: Improvement of skin wound healing for diabetic mice with thermosensitive hydrogel combined with insulin injection. *International Journal of Endocrinology*, 7847011, 2022.
- 8- Zhang X.-J., Wu X., Wolf S.E., Hawkins H.K., Chinkes D.L., et al.: Local insulin-zinc injection accelerates skin donor site wound healing. *The Journal of Surgical Research*, 142 (1): 90-96, 2007.
- 9- Abianeh S.H., Bajestani S.M., Rahmati J., Shahrabaf M.A. and Shirzad N.: The effect of local insulin injection on the healing process of split thickness skin graft donor site: a randomized, double-blind, placebo control clinical trial. *European Journal of Plastic Surgery*, 43 (5): 633-638. <https://doi.org/10.1007/s00238-020-01683-3>, 2020.

- 10- Zhao L., Niu L., Liang H., Tan H., Liu C. and Zhu F.: pH and glucose dual-responsive injectable hydrogels with insulin and fibroblasts as bioactive dressings for diabetic wound healing. *ACS applied materials & interfaces*, 9 (43): 37563-37574, 2017.
- 11- Abianeh S.H., Bajestani S.M., Rahmati J., Shahrabaf M. A. and Shirzad N.: The effect of local insulin injection on the healing process of split thickness skin graft donor site: A randomized, double-blind, placebo control clinical trial. *European Journal of Plastic Surgery*, 43 (5): 633-638, 2020.
- 12- Oryan A. and Alemzadeh E.: Effects of insulin on wound healing: A review of animal and human evidences. *Life Sciences*, 174: 59-67, 2017.
- 13- Liu Y., Dhall S., Castro A., Chan A., Alamat R. and Martins-Green M.: Insulin regulates multiple signaling pathways leading to monocyte/macrophage chemotaxis into the wound tissue. *Biology Open*, 7 (1): bio026187, 2018.
- 14- Lima M.H., Caricilli A.M., de Abreu L.L., Araujo E.P., Pelegrinelli F.F., Thirone A.C., Tsukumo D.M., Pessoa A.F.M., dos Santos M.F. and de Moraes M.A.: Topical insulin accelerates wound healing in diabetes by enhancing the AKT and ERK pathways: A double-blind placebo-controlled clinical trial. *PloS One*, 7 (5): e36974, 2012.
- 15- Unger J. and Parkin C.: Hypoglycemia in insulin-treated diabetes: A case for increased vigilance. *Postgraduate Medicine*, 123 (4): 81-91, 2011.
- 16- Sridharan K. and Sivaramakrishnan G.: Efficacy of topical insulin in wound healing: A preliminary systematic review and meta-analysis of randomized controlled trials. *Wound Repair and Regeneration*, 25(2): 279-287, 2017.
- 17- Tuvdendorj D., Zhang X.-J., Chinkes D.L., Aarsland A., Kulp G.A., Jeschke M.G., et al.: Intensive insulin treatment increases donor site wound protein synthesis in burn patients. *Surgery*, 149 (4): 512-518, 2011.
- 18- Martínez-Jiménez M.A., Valadez-Castillo F.J., Aguilar-García J., Ramírez-García Luna J.L., Gaitán-Gaona F.I., Pierdant-Perez M. and Sánchez-Aguilar J.M.: Effects of local use of insulin on wound healing in non-diabetic patients. *Plastic Surgery*, 26 (2): 75-79, 2018.