Improved Skin Wound Healing Activity of Insulin Cream as Evidenced from the Morphological Evaluation in Guinea Pigs

Nur-Aliana H. Mohamed
Centre of Clinical Science, Faculty of Dentistry, University Technology MARA, Sungai Buloh Campus, 40450 Selangor, Malaysia

Rafidah H. Mokhtar
Faculty of Medicine and Health Sciences, University Sains Islam Malaysia, 55100 Ampang, Kuala Lumpur, Malaysia

Imad M. Al-Ani
Department of Basic Medical Science, Kulliyyah of Medicine, International Islamic University Malaysia, Kuantan, Pahang 25200, Malaysia, imad_alani@yahoo.com

Azizi Ayob
School of Medicine, International Medical University, Bukit Jalil, 57000 Kuala Lumpur, Malaysia

Misni Misran
Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

Improved Skin Wound Healing Activity of Insulin Cream as Evidenced from the Morphological Evaluation in Guinea Pigs

Nur-Aliana H Mohamed[1], Rafidah H Mokhtar[2], Imad M Al-Ani[3*], Azizi Ayob[4], Misni Misran[5]

1. Centre of Clinical Science, Faculty of Dentistry, University Technology MARA, 40450 Selangor, Malaysia
2. Faculty of Medicine and Health Sciences, University Sains Islam Malaysia, 55100 Ampang, Kuala Lumpur, Malaysia
3. Department of Basic Medical Science, Kulliyyah of Medicine, International Islamic University Malaysia, Kuantan, Pahang 25200, Malaysia
4. School of Medicine, International Medical University, 57000 Kuala Lumpur, Malaysia
5. Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

*E-mail: imad_alani@yahoo.com

Abstract

Background: There is no histological study evaluating the effects of insulin-containing cream on skin injury. The goal of this study was to examine the effects of insulin-containing creams on wound healing. **Methods**: Creams consisting of nine parts of oil and one part of aqueous phase (9:1) mixed with 1.5 mL human insulin were prepared. Eighteen male guinea pigs were divided into three groups; the control (9:1 C) group received cream without insulin. The experimental groups received Humulin N (9:1 N) and Humulin R (9:1 R) respectively. A 1 cm^2 wound of 1-2 cm thickness was created in the skin. Each animal received 0.5 g of the respective creams which was topically applied once a day for 14 days. The progress of wound healing was monitored daily. Skin tissues were excised at the 14th days from the wound sites and processed for light microscopy. **Results**: Skin wound treated with the long acting insulin Humulin N had an accelerated wound healing process with restoration of vascular network, increased collagen deposition and early complete wound remodeling. **Conclusions**: Insulin cream with long acting mechanism facilitates in normalizing cell permeability, promoting vascularization, reducing exudation and stimulate proliferation of cells. These properties render insulin cream suitable for expediting wound healing.

Keywords: inflammation, insulin cream, Guinea pigs, skin, wound healing

Introduction

Wound healing is a natural restorative response to tissue injury. Healing of skin wounds is the interaction of a complex cascade of cellular events for substituting damaged and missing cellular structures and tissue layers that involves; inflammation, formation of granulation tissue, epithelial cell proliferation and regeneration, deposition of interstitial matrix, tissue remodeling and other events carried out by cells such as keratinocytes, fibroblasts, inflammatory and endothelial cell.[1,2] Numerous products with different ingredients are applied topically for treating wounds or reducing pain; most of these products are available in two types of preparations *ie* ointments or creams. The skin represents the largest and most easily accessible organ of the body and the use of topically delivered drugs via the skin provides an attractive alternative to oral administration.[3] Creams are viscous liquid or semisolid emulsions prepared for skin application; they have been used for many centuries to improve wound healing, to treat skin disease, to retard the skin aging process and preserve its natural beauty.

Creams are able to interact with both the keratin in the corneocytes and the surrounding phospholipid bilayers of the cytoplasmic membrane.[4]

Insulin is a polypeptide hormone with 51 amino acids produced by the β cells of the pancreatic islets of Langerhans. It plays a central role in regulating blood glucose levels, activating multiple substrates which have roles in various cellular processes and induce a wide variety of effects on cells signaling pathways regulating metabolic and mitogenic functions by binding to receptors on the cell surface.[5] It has been demonstrated that insulin is also involved in the proliferation and differentiation of various tissues that are not considered as classical insulin targets, this led to the possibility of insulin having effects on wound healing owing to the fact that insulin growth factor (IGF) is one of the major regulators of cellular proliferation and differentiation.[6] The role of insulin in open wound healing is still very much debated. The administration of 2.5 mU of insulin via intravenous infusion showed no improvement in skin wound healing in rabbits,[7] while the application of

0.03 IU of insulin locally for wound healing in the C57BL/6J mouse animal model showed healing via keritonocyte migration and significantly decreased the size of wounded area by day 3 after injury.[8] The application of eye drops containing insulin-like growth factor (IGF) in neonatal rats improved the corneal epithelial barrier function as compared with capsaicin-injected animals.[9] The new technological advances in chemical composition and rheological properties of creams, as well as better understanding of chemical structure of insulin, promised the possibility of developing a stable insulin cream that is able to retain its biological efficacy. A cream emulsion consisting of nine parts of oil and one part of aqueous phase (9:1) containing 1.5 mL insulin equivalent to 150 IU was prepared by Mohamed.[10] This formulation has high solid-like property and exhibit low yield stress. Using enzyme-linked immunosorbent assay (ELISA) technique, it revealed that insulin cream samples retained persistent active insulin concentrations for 30 days.[10] The role of insulin in wound healing is not well documented. However, many researches have been done to look at the potential of insulin as a wound healing agent owing to the fact that insulin increases vascularization, promotes collagen synthesis, re-epithelization and fibroblast division.[11] To date there is no known published histological study demonstrating the effects of insulin-containing cream on skin injury. Therefore, the objective of this study was to evaluate the progress of histological changes during the wound healing process after treatment with insulin-containing cream.

Methods

Chemicals. Sucrose fatty acid esters (SFAEs) were purchased from DKS Co. Ltd., Japan. Olive oil, methyl and ethyl paraben, Vaseline, and two types of surfactants Sorbitan monolaurate (Span®80) were purchased from Merck, Germany. Semisynthetic human insulin (Humulin N and Humulin R) of 100 units/mL concentration each were purchased from Eli Lily Company USA.

Preparation of insulin cream. The ratio of (9:1) of cream consisting of nine parts of oil and one part of aqueous phase was chosen. Cream components including surfactants, preservatives and the addition of 1.5 mL insulin which is equivalent to 150 IU were prepared as described by Mohamed.[10]

Animals and experimental design. Eighteen male guinea pigs weighing 400-600 g were individually housed in clean polyethylene cages under standard experimental conditions of temperature 25 °C, 12 hour light/dark cycle and fed on normal pellet diet and water ad libitum. The animals were divided into three groups of according to the cream preparation applied; (i) control group received cream without insulin (9:1 C) (n = 6); (ii) experimental group receiving long-acting insulin, Humulin N (9:1 N) (n = 6); and (iii) experimental group

receiving intermediate-acting insulin, Humulin R (9:1 R) (n = 6). The animals were treated according to the Standards and Regulations for the Care and Use of Laboratory Animals of the National Institutes of Health Malaysia and according to the guidelines of IIUM animal Ethical Committee (IIUM/519/14/4/IACUC).

Excision wound. All animal groups underwent the following excision wound. The animals were anesthetized with a combined mixture of intramuscular xylazine hydrochloride and ketamine hydrochloride at concentration of 1 cm x 1 cm. The back of the guinea pigs were shaved and a square template measuring 1 cm x 1 cm was placed on the skin. Under sterile conditions, a 1 cm^2 full thickness, both epidermis and dermis, patch of skin was removed creating an open wound. The animals in the experimental groups received 0.5 g of creams which was applied daily topically starting from the day of wound induction until Day 14. The clinical progress of wound healing was monitored daily.

Wound healing monitoring. Wound area was determined by calculating the surface area once the wound margin have been identified. The progressive changes in wound area were measured by a digital photograph uploaded to a computer with AxioVision Software, version 4.5 (Carl Zeiss, Germany). The uploaded digital image was delineated with the use of the computer mouse. The wound areas in all animals were recorded daily in cm^2. A graph of [wound area]$^{1/2}$ vs time was plotted to obtain the rate of wound contraction according to the Snowden method.[12] The reduction in size of the wound area from its original wound size was thus followed.

Histological preparation. The skin wounds were excised on Day 14 of treatment. The guinea pigs were sacrificed and the tissues from the wound site of the individual animals were removed, fixed in 10% formal saline, processed for light microscopy and sections of 5 μ thickness were stained with Haematoxylin and Eosin (H & E).

Histological grading of the wound healing response. The dynamics of the inflammatory response including formation of granulation tissue, infiltration by inflammatory cells, proliferation of fibroblasts and angiogenesis were recorded and analyzed semiquantitatively using a 4-point scale as follows: 0 - absent, 1 - weak, 2 - moderate and 3 - intense. The similar 4-point scale was used for evaluation of inflammatory cell density. The grading scale of 0 to 3 was based on the number of cells per field (objective: 20x): a Grade 1 representing 2 to 4 cells, Grade 2 representing 4 to 10 cells, and Grade 3 representing more than 10 cells.[13]

Statistical analysis. Statistical analysis was performed using one-way measurement (Analysis of variance/ ANOVA) for wound contraction rate and histological

analysis. The post-hoc test with Bonferroni test were carried out All data in this study are presented as mean ± SD. Data were analysed by Mann-Whitney U test, the value of $p < 0.05$ was considered as statistically significant.

Results

Areas of wound healing. The calculation of the [average areas of wound healing in cm^2]$^{1/2}$ vs time in the control and insulin cream formulation treated animal groups illustrates the rate of wound contraction during the period from day 1 to day 14 (Figure 1). Wound treated with 9:1 N has healing rate of 0.16114 cm per day (Table 1), which indicated that the wound treated with this cream has the highest rate of contraction.

Analysis of wound treated with creams. All induced wounds started to progressively decrease in size on different days after induction (Figure 1 and Figures 2 A, B and C). Induced wounds in group 9:1N began healing between Days 3-5 with an average healing rate of 0.162 cm^2/day as compared to 0.637 ± 0.02 cm^2 on Day 8 for group 9:1 C. On the other hand, groups 9:1 R (Figure 3 A, B, and D) and 9:1 C (Figure 3 D, E, and F) showed healing rates of 0.12631 cm^2/day and 0.11335 cm^2/day respectively (Table 1). Post Hoc analysis revealed that induced wounds in group 9:1N healed significantly faster (R = -0.99, $p < 0.002$) than 9:1R and 9:1C. Interestingly, there was no significant difference in wound healing rates between animals treated with 9:1 R and 9:1 C.

Histological healing response with time. The correlation between the rate of healing response and time is presented in Table 2. Among all cream preparations the 9:1 N had the strongest correlation coefficient with R= -0.99 ($p < 0.002$).

Histological findings. During the post-surgery period, the animals remained healthy, without clinical evidence of infection. Results are analyzed by one-way ANOVA to compare the mean ± SE for all histological parameters such as inflammatory cells, fibroblasts, granulation tissues and angiogenesis. Table 3 demonstrates the analysis of histological findings showing the neutrophils, lympho-cytes and macrophages count were all significantly decreased in 9:1 N cream group as compared to the control group.

Robust inflammatory response accompanied the wounded skin layers, in which first neutrophils and then macro-phages and lymphocytes were observed that emigrated from nearby tissues and from the circulation. The intense inflammatory reaction in wounds treated with 9:1 N cream preparation was no longer observed on day 14 when it was replaced by organized tissue with weak
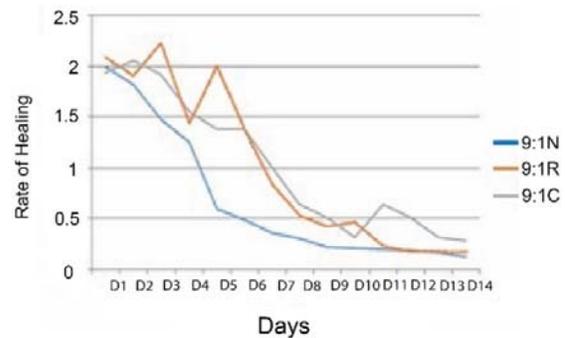
Figure 1. Graph showing rate of healing in wound treated with different formulations

Table 1. Rate of healing of wound treated cream. Note that the rate is highest in 9:1 N application

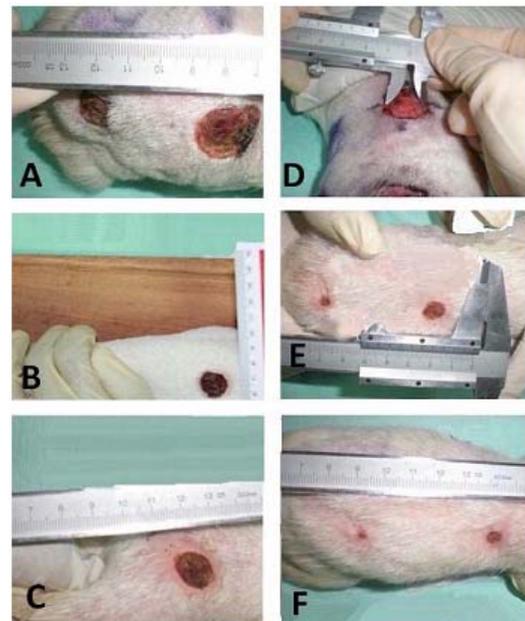| Type of cream	N	Rate of healing (cm/day)
9:1 N	6	0.16114
9:1 R	6	0.12631
9:1 C	6	0.11335

Figure 2. A, B, C. Morphological Observations of Wound Treated with Insulin Creams Showing Wounds Started to Decrease on Different Days. Wound Area Decreased in 9:1 N Group "A" on Day 5 (0.590 ± 0.21 cm2), in 9:1 R Group "B" on Day 7 (0.838± 0.43 cm2) and in 9:1 C Group "C" on day 8 (0.637± 0.02 cm2). D, E, F. Wound Treated with 9:1 N on Day 1, 9 and 14. Note that Wounds Treated with 9:1 N Showed Almost Complete Healing Process and had Reached Tissue Remodelling on Day 14 as Compared to other Treated Groups.

Figure 3. A, B, C. Wound Treated with 9:1 R on Day 1, 9 and 14. Note that the Representative Wound Still in Inflammatory Phase on Day 14 as Compared to Wound Treated with 9:1 N. D, E, F. Wound Treated with 9:1 C on Day 1, 9 and 14. Note that Wound Treated with 9:1 C was in Inflammatory Phase as Compared to Wound Treated with 9:1 N

Table 2. The Linear Correlation Coefficient (R) of the 9:1 Cream Indicate How Strong the Relationship between Wound Area and the Time Observed

Type of cream	N	R	*p*
9:1 N	6	-0.99	0.002
9:1 R	6	-0.94	0.025
9:1 C	6	-0.92	0.043

Note: All creams have a significant negative correlation between rate of healing and time factor. Among all cream prepared, 9:1 N has the strongest correlation coefficient with R= - 0.99 ($p < 0.002$).

cells in wound treated with different creams. Furthermore, the mean of inflammatory cells are shown in Figure 5.

Table 3. Semiquantitative Analysis of Inflammatory Cells in Different Creams Via ANOVA

Inflammatory cells	Creams	Mean ± SE	F statistic (df)	*p*
Neutrophils	9:1 N	1.17±0.17	26.73 (5, 30)	0.026*
	9:1 R	3.17 ± 0.22		
	9:1 C	2.67± 0.21		
Lymphocytes	9:1 N	1.67 ± 0.33	19.76 (5, 30)	0.008*
	9:1 R	3.23 ± 0.17		
	9:1 C	3.33 ± 0.33		
Macrophages	9:1 N	1.50 ± 0.37	10.68 (5, 30)	0.001*
	9:1 R	3.53 ± 0.12		
	9:1 C	3.50 ± 0.22		

Note: *Significance differences in all inflammatory cells count between all creams ($p < 0.05$).
Not that all neutrophils, lymphocytes and macrophages count were significantly decrease in 9:1 N cream.
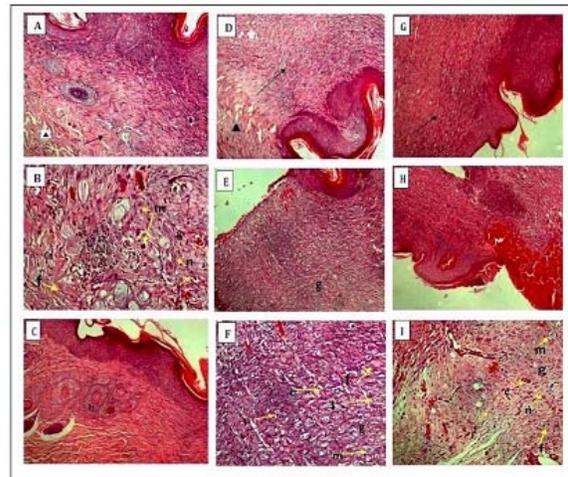

Figure 4. Histopathology Study of Wound Treated with Insulin Containing Cream. A, B, C Treated with 9:1; N; D, E, F Treated with 9:1 R; G, H, I Treated with 9:1 C. Notice the more Progressive of Wound Healing in 9:1 N Group than Both 9:1 R and 9:1 C. Hair Follicles (h) were Demonstrated in 9:1 N Group. The Black Arrow Indicates the Healing Area. The Normal Tissue is Indicated as (Head Arrow); Collagen (Co) and Fibroblasts (f) Located Longitudinally to the Wounds. Note that the Presence of Granulation Tissues (g) with Less Intense Inflammatory Cells Namely the Neutrophils (n), Lymphocytes (l) and Macrophages (m) were Demonstrated Fibroblasts (f) Dispersed in the Wound of 9:1 C is more Obvious than Wound Treated with 9:1 R with Considerable Number of New Capillaries (c)
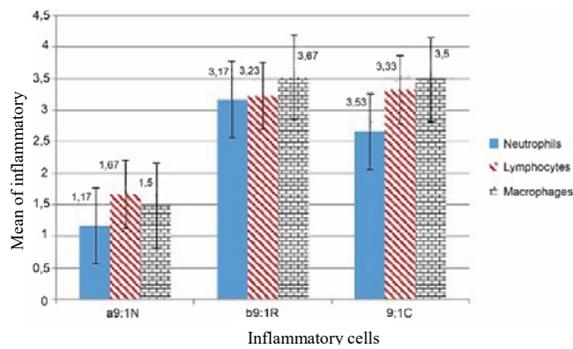
presence of inflammatory cells (Figure 4 A, B and C). Although inflammatory cells were found in wounds treated with 9:1 N cream but not as prominent as in 9:1 R (Figure 4 D, E and G). Inflammatory cells were also seen in 9:1 C treated wounds (Figure 4 E, F and G), where neutrophils were dispersed in moderate numbers in the granulation tissue together with macrophages and lymphocytes. It is observed that 9:1 R demonstrated the highest density of inflammatory cells as compared to all treatment groups. Neutrophils, macrophages and lymphocytes concomitantly invaded the wound area (Figure 4 D, E and G). Table 3 demonstrates the inflammatory

Figure 5. Graph on Mean ± SD of different inflammatory cells. Bars show the means, error bars shows standard error

Discussion

The healing process of the wounds in the two groups of insulin cream treated animals and a control group with experimentally inflicted skin wounds was studied to observe the variability of the inflammatory and proliferative tissue responses and wound healing. The histopathology of acute inflammatory phase in guinea pigs was not described in this study. However, the tissue proliferation and wound repair in each experimental group were observed. Wound healing of injured dermis has been described to run in three basic phases, inflammation, proliferation and maturation.[14] The present study has demonstrated the healing of full thickness wounds; the results of wound area measurements in the current study indicated healing potential with application of intermediate acting insulin cream. In the early inflammation phase; it showed that all treated wounds demonstrated increased of wound area after a few days of wound induction. This might be due to normal homeostasis mechanism which is initiated by prostaglandins released from injured tissue and histamine from the mast cells induce vascular dilatation with increased capillary permeability.[15] Leaking of plasma through the permeable vessels in the surrounding tissue causes site of the injury to become swollen; at this point, all inflammatory cells actively invaded the wound area.[16] This study concurred with the increase in wound size due to inflammation homeostasis which is regulated by physiology of the tissue as well the healing mechanism triggered by inflammatory cells such as neutrophils, lymphocytes and macrophages.

The phase II of wound healing constitutes tissue proliferation and repair mechanisms which leads to covering the wound surface with new skin. The morphological findings of the present study demonstrated that wound treated with 9:1 N has a high rate of wound contraction; these effects were highlighted by the full thickness coverage of the wound area with organized epidermis in the presence of mature scar tissue in the dermis. Further-

more 9:1 N cream may have potential in restoring vascular integrity to the region and repairing the structure integrity of the tissue defect by filling it with new connective tissue. Histopathological observations supported the morphological findings of wound treated with 9:1 N; some of the tissue sample even reached remodelling stage on day 14. Granulation tissues gradually ceased and fibroblasts count was also decreased in 9:1 N. Fibroblasts significantly decreased in number when homeostasis signalled the onset of maturation phase. The process of restoring the vascular network is called neovascularization or angiogenesis around the wound surface.[17] Angiogenesis was present in all treated groups. It occurred concurrently with fibroblast proliferation when endothelial cells migrate to the area of the wound. However, in wound treated with 9:1 N, mature blood vessels were observed with some tissue samples showing complete wound remodelling. Wound contraction is mediated by specialized fibroblasts "myofibroblasts" and reorganization of the extracellular matrix which is found within the granulation tissue, where they carry out the important process after initial injury of providing mechanical support and integrity to the tissue.[18,19] This study demonstrated significant increase in fibroblasts and reformation of collagen fibres at day 14 of treatment with 9:1 N. One of fibroblasts most important function is the production of collagen, which is newly synthesized in the site of wound healing and occupies a central role in wound healing and provides structural framework, strength and milieu for the generating tissue.[19] The present study found that wounds treated with 9:1 R have higher rate of contraction and healed faster than wounds treated with 9:1 C cream. Inflammatory cells infiltrates were found at significantly higher numbers in wounds treated with insulin R than in insulin N treated groups.

The major innate immunity cells that reach the site of injury are neutrophils and macrophages, they play a critical role in tissue development, homeostasis, and injury repair.[20] Neutrophils initiated wound repair by activating local fibroblasts and epithelial cells and removing the invading microbes and cellular debris in the wound area; neutrophils are usually predominant for the first few days and then disappear.[21] However, it was found that neutrophils predominated in some of the wounds in this study especially in the 9:1 R group whereby the healing process was delayed. This implied that 9:1 R cream is not effective in promoting wound healing as compared to 9:1 N. In the absence of infection, the existing monocytes differentiate into macrophages and become the major phagocytic cells at the injury site.[20] Macrophage infiltration predominates for the wound healing process, and allow for harnessing regenerative functions over inflammatory functions of myeloid cells.[22] In the present investigation, the number of macrophages significantly increased in wound treated with 9:1 R and 9:1 C. The macrophage has an essential role in wound healing, it synthesizes a variety of

cytokines including growth factors involved in the migration, proliferation, and organization of new connective tissue and vascular beds within the wound.[23]

Wound contraction, ie the process of wound area shrinkage, depends on the reparative abilities of the tissue, type and extent of the damage and general state of health of the host.[24] The results of this study supported that wound healing and repair were accelerated by applying 9:1 N. Thus, one might postulate that it exerted its observed action as a result of the more persistent presence and binding of the longer acting insulin N component of 9:1 N to its respective receptors.

The final stage of wound healing is remodeling which involves a series of events in an attempt to recover the tensile strength and normal tissue structure through reorganization, degradation, and re-synthesis of the extra-cellular matrix forming scar tissue.[1] Wound remodeling may begin at different times and different region.[25] In tissue remodeling stage, extracellular matrix bound growth factors and metalloproteinases (MMPs) are activated by macrophage and fibroblasts, resulting in degradation of the matrix and differentiation of cells.[26] In the present study, only wound treated with 9:1 N showed significant maturation of granulation tissue and remodeling on Day 14.

Conclusions

In conclusion, the present investigation gives credence to the hypothesis that insulin cream formula has wound healing potential and that the Humulin N induces faster wound healing process than Humulin R in the guinea pig animal model and thus it may have a promising prospect in the management of skin wounds after further clinical testing in the future.

Funding

Conflict of Interest Statement

The authors declare that they have no personal or financial conflict of interest.

References

1. Maxson S, Lopez EA, Yoo D, Danilkovitch-Miagkova A, Leroux MA. Concise review: role of mesenchymal stem cells in wound repair. *Stem Cells Transl Med*. 2012;1:142-9.
2. Mesher AL. Macrophages and fibroblasts during inflammation and tissue repair in models of organ regeneration. *Regeneration*. 2017;4:390-53.
3. Chen J, Gao Y. Strategies for meloxicam delivery to and across the skin: a review. *Drug Deliv*. 2016;23:3146-56.
4. Frankel A, Sohn A, Patel RV, Lebwohl M. Bilateral comparison study of pimecrolimus 1% cream and a ceramide-hyaluronic acid emollient foam in the treatment of patients with atopic dermatitis. *J Drugs Dermatol*. 2011;10:666-72.
5. Ramalingam L, Oh E, Thurmond DC. Novel roles for insulin receptor (ir) in adipocytes and skeletal muscle cells via new and unexpected substrates. *Cell Mol Life Sci*. 2012;70:2815-34.
6. Sadagurski M, Yakar S, Weingarten G, Holzenberger M, Rhodes CJ, Breitkreutz D, *et al*. Insulin-Like growth factor 1 receptor signaling regulates skin develop-ment and inhibits skin keratinocyte differentiation. *Mol Cell Biol*. 2006;26:2675-87.
7. Xiao-Jun Z, Chinkes DL, Irtun O, Wolf SE. Anabolic action of insulin on skin wound protein is augmented by exo-genous amino acids. *Am J Physiol*. 2002;282:E1308-15.
8. Liu Y, Petreaca M, Yao M, Martins-Green M. Cell and molecular mechanisms of keratinocyte function stimulated by insulin during wound healing. *BMC Cell Biology*. 2009;10:1-15.
9. Eguchi H, Hiura A, Nakagawa H, Kusaka S, Shimumora Y. Corneal Nerve Fiber Structure, Its Role in Corneal Function, and Its Changes in Corneal Diseases. *BioMed Res Int*. 2017;2017:3242649.
10. Mohamed NAH. Novel insulin cream preparation in managing wound healing [Thesis]. Kuantan: International Islamic University Malaysia; 2009.
11. Gomes A, Teixeira C, Ferraz R , Prudêncio C, Gomes G. Wound-Healing peptides for treatment of chronic diabetic foot ulcers and other infected skin injuries. *Molecules*. 2017;22:1743.
12. Snowden JM. Wound contraction: A quantitative inter-pretation. *Australian Biol Med Sci*. 1981;59:203-15.
13. Todorovic V, Pesko P, Micev M, Bjelovic M, Budec M, Micic M, *et al*. Insulin-like growth factor-I wound healing of rat skin. *Regul Pept*. 2008;150:7-13.
14. Gonzalez AD, Costa TF, Andrade ZA, Medrado ARAP. Wound healing - A literature review. *An Bras Dermatol*. 2016;91:614-20.
15. Kalogeris T, Baines CP, Krenz M, Korthuiz RJ. Cell biology of ischemia reperfusion injury. *Int Rev Cell Mol Biol*. 2012;298:229-317.
16. Aller MA, Arias JI, Arias J. Pathological axes of wound repair: Gastrulation revisited. *Theor Biol Med Model*. 2010;7:1-32.
17. Kanji S, Das H. Advances of Stem Cell Therapeutics in Cutaneous Wound Healing and Regeneration. *Mediators Inflam*. 2017;2017:5217967.
18. André-Lévigne D, Modarressi A, Pepper MS, Pittet-Cuénod B. Reactive oxygen species and NOX enzymes are emerging as key players in cutaneous wound repair. *Int J Mol. Sci*. 2017;18:2149.
19. Darby IA, Laverdet B, Bonté F, Desmouliere. Fibroblasts and myofibroblasts in wound healing. *Clin Cosmet Investig Dermatol*. 2014;7:301-11.
20. Ogle ME, Segar CE, Sridhar S, Botchwey EA. Monocytes and macrophages in tissue repair: Implications for immunoregenerative biomaterial design. *Exp Biol Med*. 2016;241:1084-97.
21. Selders GS, Fetz AE, Radic MZ, Bowlin GL. An overview of the role of neutrophils in innate immunity,

inflammation and host-biomaterial integration. *Regen Biomaters*. 2017;4:55-68.

22. Awojoodu AO, Ogle ME, Sefcik LS, Bowers DT, Martin K, Brayman KL. Sphingosine 1-phosphate receptor 3 regulates recruitment of anti-inflammatory monocytes to microvessels during implant arteriogenesis. *Proc Natl Acad Sci USA*. 2013;110: 13785-90.

23. Serra MB, Barroso WA, Neves da Silva N, Borges ACR, Abreu IC, Borges MODR. From inflammation to current and alternative therapies involved in wound healing. *Int J Inflam*. 2017;2017:3406215.

24. Patil MV, Kandhare AD, Bhise SD. Pharmacological evaluation of ethanolic extract of Daucus carota Linn root formulated cream on wound healing using excision and incision wound model. *Asian Pac J Trop Biomed*. 2012;2:S646-55.

25. Strodtbeck F. Physiology of wound healing. *Newborn and Infant Nurs Rev*. 2011;1:43-52.

26. Olczyk P, Mencner A, Komosinska-Vassev K. The role of the extracellular matrix components in cutaneous wound healing. *Bio Med Res Int*. 2014;2014:747584.