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The Effect of Secretome Hypoxia Mesenchymal Stem Cells on PDGF and IL-1b Gene Expression (Experimental Study on Wistar Rats Hyperglycemic Wound Models)

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ABSTRACT: Hyperglycemic wounds take longer to heal, and high glucose results in an increase in inflammatory cells, a decrease in the angiogenesis process, and the production of growth factors. Antibiotic treatment, surgery, or debridement can increase the risk of amputation. This study aimed to determine the effect of administering gel secretome hypoxia mesenchymal stem cells (SH-MSCs) on the expression of PDGF and IL-1b in Wistar rats with a hyperglycemic wound model. In vivo laboratory experimental research with a Post Test Only Control Group Design. The samples consisted of 30 Wistar rats, divided into 5 groups, namely healthy rats (P1), negative control (P2), positive control with gentamicin (P3), gel secretome dose of 20 µL/rat (P4), and gel secretome dose of 40 µL/rat (P5). MSC secretome hypoxia gel treatment for 10 days, then skin tissue samples were examined using the RTq-PCR method to analyze PDGF and IL-1b gene expression. Analysis of PDGF gene expression showed significant differences between treatment groups using the Kruskal-Wallis test with a result of 0.018 ($p<0.05$), but there were no significant differences between groups using the Mann-Whitney test ($p<0.05$). Analysis of IL-1b gene expression showed significant differences between treatment groups using the Kruskal Wallis test with a result of 0.001 ($p<0.05$), various doses of secretome gel affected reducing IL-1b gene expression using the Mann Whitney test with a result of 0.004 (p<0, 05). The most significant decrease was at a secretome dose of 40 µL/mouse. MSC secretome hypoxia gel at a dose of 40 µL/rat effectively reduces IL-1b gene expression in Wistar rats with a hyperglycemic wound model. However, various doses of MSC secretome hypoxia gel do not significantly increase PDGF gene expression.

KEYWORDS: PDGF, IL-1b, SH-MSCs, hyperglycemic wounds

I. INTRODUCTION

Poor glycemic control in the long term negatively affects wound recovery.[1] Hyperglycemic wound conditions take longer to heal.[2] The difficulty of wound healing is caused by high glucose in the microenvironment and various biological factors. Wounds are easily damaged, difficult to heal, and eventually develop into chronic wounds.[1] Wound healing becomes inhibited due to an increase in inflammatory cells, a decrease in the process of angiogenesis, and a decrease in growth factor production.[3] Hyperglycemia is known to induce the expression of interleukin 1b (IL-1b) in several different cell types in each injury event. The IL-1b pathway can be part of a positive feedback loop that maintains inflammation in chronic wounds and contributes to impaired healing.[4] Platelet-derived growth factor (PDGF) is a growth factor that regulates cell growth and division, PDGF promotes wound healing by strengthening chemotactic migration, mitosis, and proliferation of inflammatory cells and fibroblasts to promote the formation of granulation tissue in wounds.[5]

Treatment of hyperglycemic wounds with antibiotics, surgical or debridement, can still increase the risk of amputation with frequent recurrences.[6] Growth factor therapy is an interesting strategy to accelerate wound closure, MSCs are multipotent stromal cells that can regenerate tissue damage.[7] Therefore, an effective and safe adjunct therapeutic approach is needed for wounds, one of which is mesenchymal stem cells (MSC) secretome hypoxia that can polarize into anti-inflammatory cytokines in a hypoxic environment and produce various anti-inflammatory cytokines and growth factors.[8]

Wound healing is disrupted due to complications of the disease that reduce physical activity and cause chronic wounds up to amputation.[9] Hyperglycemia in patients with diabetes, vascular disease, and neuropathy is a condition that hinders the wound healing process.[4] Wound conditions in hyperglycemia are linked to complications of diabetes mellitus, affecting 439 million people globally by 2030 (aged 20 to 79 years), potentially causing chronic ulcers and increasing the risk of amputation 17 to 40 times higher.[10]

Research shows that MSC secretome hypoxia secretes various mediators and growth factors that promote skin regeneration, namely PDGF, Hepatocyte growth factor (HGF), Vascular endothelial growth factor (VEGF), Fibroblast growth factor (FGF) and Transforming growth factor-β 1 (TGF-β1). The hypoxia secretome has anti-inflammatory potential and has the ability to detect the presence of injury signaling molecules, migrate to the injury area and accelerate wound healing.[11] In Vitro research shows that MSC secretome activates the Phosphatidylinositol 3-kinase (PI3K/Akt) signaling cascade or Focal adhesion kinase mediated signaling (FAK)/ Extracellular regulated kinase (ERK1/2) and further increases the proliferation and migration ability of various skin cell types, such as fibroblasts, keratinocytes, and vascular epithelial cells thereby accelerating contraction wound, inhibition of this signaling pathway resulting in beneficial effects induced by secretions on various skin cells.[12] Other preclinical studies reported routine antioxidant and anti-inflammatory effects on wound healing in streptozotocin-induced hyperglycemic rats. Rutin increases the production of antioxidant enzymes induced by nuclear erythroid related factor 2 (NRF2), inhibits the expression of matrix metalloproteinases (MMPs) regulated by NF-κB, and decreases the expression of vascular endothelial growth factor (VEGF), reduces oxidative stress and inflammatory response in hyperglycemic rats thereby improving wound healing and further reducing the risk of ulcer development.[1]

PDGF at a concentration of 20-200 pmol accelerates the infiltration of inflammatory cells and fibroblasts, extracellular matrix deposition, and collagen formation in wounds, resulting in a rapid healing process compared to untreated models.[13] In Vivo studies report that several factors may influence the phenotype of macrophages expressed in chronic wounds, having the potential to maintain proinflammatory environment. Macrophages in chronic wounds express high levels of proinflammatory molecules IL-1b, matrix metalloproteinase 9 (MMP-9), tumor necrosis factor-alpha (TNF-α), and decreased expression of nonprohealing markers insulin-like growth factor 1 (IGF-1), transforming growth factor beta (TGF-β), and interleukin 10 (IL-10). In hyperglycemic conditions known to induce IL-1b interleukin expression in a number of different cells, the IL-1 pathwayβ being part of a positive feedback loop that sustains inflammation in chronic wounds and contributes to impaired healing.[4]

MSC secretome hypoxia can suppress the secretion of pro-inflammatory cytokines thereby lowering levels of IL-6, IL-1b, TNF-α, IL-4 and accelerating the improvement of wound conditions in mice.[14] Research reveals MSC secretome hypoxia secretes growth factors that support skin regeneration, such as PDGF regulates cellular activity in the wound healing process, detects the presence of injured signal molecules, migrates to the injured area and accelerates wound healing.[3,11] Various studies report the potential of the precondition MSC secretome in repairing wound tissue, enhancing regenerative properties in cell secretion. MSC secretome hypoxia as a source of tissue protective and regenerative secretions in wound healing.[15] However, the potential of MSC secretome hypoxia gel in regenerating hyperglycemic wounds has never been done. Based on this background, it is necessary to conduct research to determine the effect of MSC secretome hypoxia gel on the expression of PDGF and IL-1b genes in male Wistar rat hyperglycemic wound models.

II. MATERIAL AND METHOD

Study Design and Experimental Animals

This research is a true research of laboratory experiments in vivo with the design of Post Test Only Control Group Design. The study subjects used male white rats of the Wistar strain, ranging in age from 2-3 months with a weight between 200-250gr which met the criteria of inclusion and inclusion, acclimatized for 7 days. This study used 5 treatment groups, a group of healthy mice (P1), a group of negative control rats of hyperglycemic wound model with base gel administration (P2), a positive control group of hyperglycemic wound rat models with standard therapy using gentamicin antibiotics (P3), a group of hyperglycemic wound rat model with administration of MSC secretome hypoxia gel dose of 20 µL / rat (P4), and a group of hyperglycemic wound model with MSC gel administration secretome hypoxia dose of 40 µL/rat (P5).

Research Materials

This research uses equipment consisting of 1 cc injection syringe, balance sheet, handscoon, gluco test easy touch, stemple mold wound 2x2 cm tweezers, Handscoon surgical scissors, sterile swab, biosafety Cabinet (BSC), Oxygen meter, beaker glass, stirring rod, micropipette, CO2 incubator, Dissecting kit, Flask 75T, Hypoxic chamber.

Research Equipment

This research uses equipment consisting of a Biosafety Cabinet (BSC), micropipette, CO2 incubator, Dissecting kit, Flask 75T, Hypoxic chamber, oxygen meter, beaker glass, stirring rod, 1 cc injection syringe, balance sheet, Gluco test easy touch, tweezers, surgical scissors, handscoon, and sterile swab.

MSC Isolation from Umbilical Cord

The isolation stage is carried out in biosafety cabinet class 2, with aseptic and sterile-conditioned tools. The steps are as follows: (1) Collect the Umbilical cord then place it in a sterile container containing 0.9% NaCl. (2) The Umbilical cord is placed on a petri dish, and then washed thoroughly with PBS using tweezers. (3) Cut the umbilical cord, separate it from the rat fetus, and remove the blood vessels. (4) Finally chop the umbilical cord, insert it into the 25T flask then flatten, and let stand for 3 minutes until the tissue sticks to the surface of the flask. (5) Complete media (DMEM, fungizone, penstrep, and FBS) are added slowly until the network is covered with media. (6) Incubation with an incubator at 37°C and 5% CO2. (7) Cell growth is detected about 14 days after the culture process, then add media every 3 days by removing half of the media and completely replacing it with new media. (8) The addition of cells is repeated so that the cells reach 80% confluence.

Hypoxia and TFF Manufacturing

(1) MSC that has reached 80% confluence plus complete media up to 10 mL (2) Flask containing MSC is inserted into the hypoxic chamber. (3) Nitrogen gas is flowed through the inlet valve and the oxygen meter is placed in the sensor hole to determine the oxygen concentration. (4) Nitrogen is then added until the oxygen concentration is 5%. (5) After 24 hours, culture media will be removed and screened using TFF to obtain hypoxia MSC secretome and then will be used on P4 and P5.

Preparation Manufacturing Gel MSC Secretome Hypoxia

(1) MSC secretome hypoxia gel preparation is prepared by mixing 20 µL secretome added 20 µL in 200mg gel so as to obtain a concentration of 20 µL/rat (P4). The MSC secretome hypoxia gel preparation for (P5) was prepared by mixing 40 µL of secretome with 20 µL added in 200 mg of gel to obtain a concentration of 40 µL/rat. (2) Aseptically mix until homogeneous based on physical properties observed using a microscope.

Hyperglycemic Mouse Model Making

(1) Rats were acclimatized for 7 days, then satisfied for 8-12 hours, then anesthetized with a mixture of ketamine (60 mg/kgBW) and xylazine (20 mg/kgBW) and given intraperitoneal doses using streptozotocin 65 mg/kgBW/day dissolved in 0.1 M citrate buffer pH 4.0 for 30 days. A blood glucose test is performed on day 7 after initiation. The diagnosis of hyperglycemia is established when blood glucose levels >200 mg/dl. (2) After 30 days of Streptozotocin injection, rat hair was shaved on the back. Making wounds using a circular biopsy punch diameter of 6 mm with a depth of 2 mm.[59]

Validation of Hyperglycemic Wound Examination

Microscopically, skin samples were taken from all groups to make histopathological preparations with hematoxylin-eosin (HE) staining. (1) Skin samples of mice of all groups were taken and fixed in 10% NBF solution, Rinse with 70% alcohol until free of fixative residue. (2) Skin samples were dehydrated with 70%, 80%, 90%, 96% alcohol, and absolute alcohol for 30 minutes each. (3) The sample is soaked with toluol for 1 hour until clear or transparent. (4) In the oven, the paraffin infiltration process is carried out at a temperature of 56-60°C by placing the skin sample in a mixture of toluol and paraffin with a ratio of 3: 1, 1: 1 and 1: 3 for 30 minutes. Skin samples were placed on pure paraffin I, pure paraffin II, and pure paraffin III for 30 minutes at each treatment. (5) Embedding process, a sample of pure paraffin skin is implanted into a paraffin block mold containing liquid paraffin and wait for it to harden, and then the paraffin block is cut to a thickness of 6 µm with microtomes. (6) Slices of skin tissue samples are attached to a glass object by applying Mayer's albumin and then dripped with a little distilled water and then heated using a hot plate until the slices stick firmly. (7) The histological preparation is deparafinisation by placing it on xylol for 24 hours. (8) Continue with HE staining. The xylol content is taken with filter paper, then added in alcohol 96%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, and aqueous is done for 1-2 minutes. The preparation is placed on the HE dye for 5-10 seconds, then rinse under running water for 10 minutes. The next process is to soak the preparation using 30%, 40%, 50%, 60%, and 70% alcohol for 3-5 minutes. The preparation is placed in eosin for 5-10 minutes, then soaked in 70%, 80%, 90%, and 96% alcohol for 3-5 minutes, and dried with filter paper. The preparation is put into xylol for 15 minutes, then the histological preparation is given drops of Canada balsam. (9) The preparation is covered with a cover glass, given etiquette, and stored in the preparation box.[60]

Hyperglycemic Wound Care

Hyperglycemic wounds formed on the sixth day, then rats were treated for 10 days with daily administration of MSC secretome hypoxia gel, with a composition of MSC secretome hypoxia gel dose 20 µL/rat (P4) and dose 40 µL/rat (P5). The positive control mice (P3) received standard antibiotic therapy gentamicin, and the negative control mice received base gel treatment (P2).

RNA Isolation Process and cDNA Synthesis

(1) A 100 mg skin tissue sample is cut into small pieces and inserted in a tube containing 0.5 mL of Iso Plus RNA. (2) Skin sample pieces were smoothed with micropestle with RNAse free DNA added 0.5 mL of Iso Plus RNA and then stored at room temperature for 5 minutes. Add 2 mL of chloroform and stir the solution until milky white using a vortex mixer. (3) Incubation for 2-3 minutes at room temperature and centrifugation for 15 minutes at 4°C at a speed of 15,000 rpm until three layers are obtained. (4) The topmost layer containing mRNA is transferred into the microtube and isopropanol is added in equal proportions. (5) Shake the microtube until white fibers appear, then centrifuge at 4^0 C at 15,000 rpm for 10 minutes. The supernatant is discarded and the remaining white pellets appear at the bottom of the tube. After drying, add 100 mL of 70% ethanol to the DEPC (Diethyl pyrocarbonate) solution flip back several times then centrifuge again at 40 C, 15,000 rpm for 5 minutes. (6) Remove the supernatant and add DEPC 30-50 µL, then as much as 5 µL of RNA sample is quantified using Nanodrop with a wavelength of 260 nm. The quantification result will be calculated to make 3000 ng. (7) The process of cDNA synthesis by mixing as much as 1 µL of RNA samples that have been calculated previously with 1 µL of OligoDT and Nuclease Free Water (NFW) primers until reaching a volume of 10 µL, then inserted in a thermal cycler for 5 minutes at a temperature of 70°C. (8) Add 5X buffer 4 µL, DEPC-Treated H_2O 5 µL, ReverTraAce 1 µL. (9) The mixture is incubated at 45-50° C for 30 min.[61]

Diagnosis of PDGF and IL-1b Gene Expression RTq-PCR method

(1) PDGF and IL-1b gene expression were analyzed using RTq-PCR with a mixture of 1 µL cDNA sample each, KAPA SYBR FAST qPCR Master Mix as much as 10 µL, primer forward and reverse as much as 1µL each, and nuclease-free water up to a total of 20 µL. (2) PCR gene expression was analyzed using qRT-PCR illumine with a predenaturation temperature profile of 95o C for 2 minutes, denaturation of 95°C for 30 seconds, then aneling for 20 seconds at a temperature of 61°C with a cycle of 50 times. (3) Increased gene expression was analyzed in the ratio of improvement to gene housekeeping using EcoStudy software.[62]

III. RESULT

Isolation of MSC Secretome from Umbilical Cord

The hypoxia MSC secretome used in this study was the result of filtration of MSC culture medium under 5% hypoxia conditions using Tangential Flow Filtration (TFF) for 24 hours then MSC was isolated from the umbilical cord of Wistar rats in a 19-day bunting state, the addition of media consisting of DMEM (Dulbeccos's modified eagle medium), fungizone, penstrep, and FBS, the results of isolation was then cultured in a plastic flap. MSC validation is performed after the 4th phase. Microscopic examination of MSC

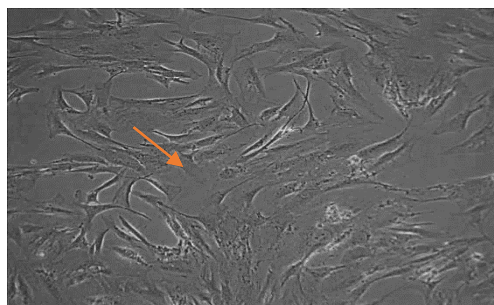

Figure 1. MSC isolation with spindle-like cells

culture results at 80% confluenc
e showed cells attached to the base of the flask with spindle-like cell shapes (Figure 1).

Examination of the capacity of MSCs to differentiate into osteogenic and adipogenic cells serves to validate the results of MSC cell isolation procedures. Oil red O staining is used in adipogenic differentiation assays to show the formation of red lipid droplets (Figure 2.a). The osteogenic differentiation assay of Alizarin red staining shows the production of calcium disposition, which is seen in red (Figure 2.b).

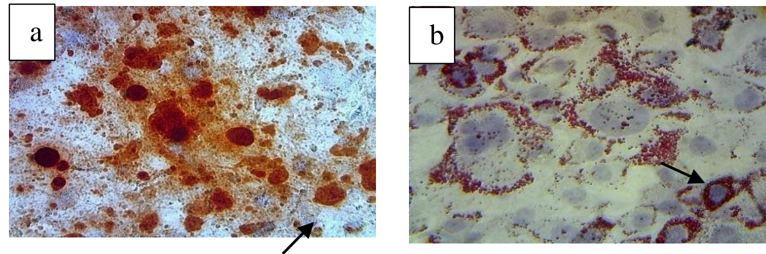

Figure 2. (a) MSCs differentiate into osteocytes and (b) MSCs differentiate into Adipocytes

Validation with flow cytometry, the results of MSC secretome hypoxia isolation show the capacity to express various special surface markers (surface markers). MSC expresses CD90 (97.6%), CD29 (96.4%) and expresses CD45 (1.7%), and CD31 (3.9%), the results of flow cytometry analysis are shown in Figure 3:

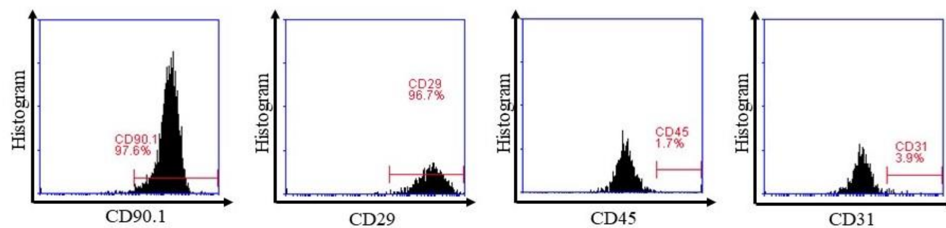

Figure 3. Flow cytometry analysis of CD90, CD29, CD45 and CD31 expression

MSC secretome hypoxia is suspected to contain anti-inflammatory cytokines and growth factors, therefore, after the filtration process is carried out analysis of the content of anti-inflammatory cytokines and growth factors, measurement of growth factor content in MSC secretome hypoxia is carried out using the ELISA test. MSC secretome hypoxia is known to produce PDGF levels of 947.26 ± 34.93 pg/mL, VEGF levels of 1137.56 ± 37.51 pg/mL, bFGF levels of 1175.54 ± 38.82 pg/mL, IL-10 levels of 525.12 ± 10.24 pg/mL, and TGF-β levels of 372.53 ± 9.18 pg/mL.

Validation of Blood Sugar Levels in Hyperglycemic Model Rats

Validation examination of glucose levels in research subjects on day 30, 36, and 46, to ensure the condition of hyperglycemia in each study subject according to the inclusion criteria for hyperglycemic wound models. The results of the glucose level examination are described in Table 1.

Table 1. Results of glucose level validation in hyperglycemic group rats

Group	P1 H0	P1 H30	P1 H36	P1 H 46	P3 46	P4	P5
Mean	105.7	419.3	388.2	382	311.8	304	294
SD	6.19	90.28	74.41	57.48	45.2	29.19	51.11

Average glucose levels in the 30th-day hyperglycemic treatment group showed a significant increase in the results of the healthy group of 105.7 ± 6.19 increased significantly to 419.3 ± 90.28, the average level of the 36th-day hyperglycemia group obtained results of 388.2 ± 74.41, the average levels in the 46th day hyperglycemic group obtained results of 382 ± 57.48, compared to the gentamicin group 311.8 ± 45.2, secretome group 20 µL 304 ± 29.2 and secretome group 40 µL 294 ± 51.1.

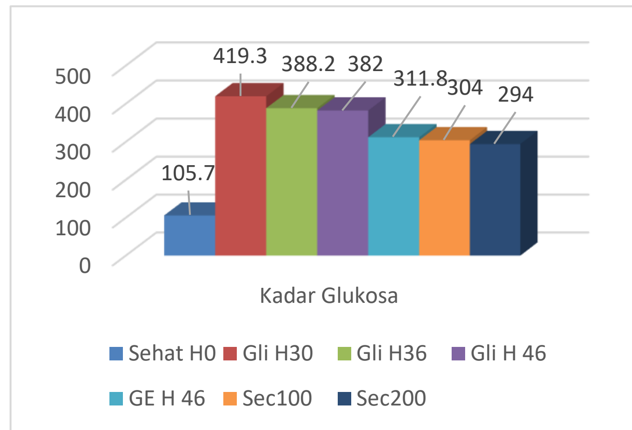

Figure 4. Results of glucose level validation in treatment group rats

The results of glucose levels showed a significant increase in the hyperglycemic group on day 30, 36, and 46 so this hyperglycemic condition can be modeled as hyperglycemic wounds.

Macroscopic Picture of Hyperglycemic Wounds

The results of macroscopic observational studies on hyperglycemic wounds on day 36, 43, and 46, showed accelerated wound healing using secretome. Using the 40 µL/rat secretome most macroscopically significant, on day 46 wound healing was visibly camouflaged and covered with growing hair, indicating that the use of secretome is effective in accelerating wound healing, the results of macroscopic wound closure analysis are shown in Figure 5.

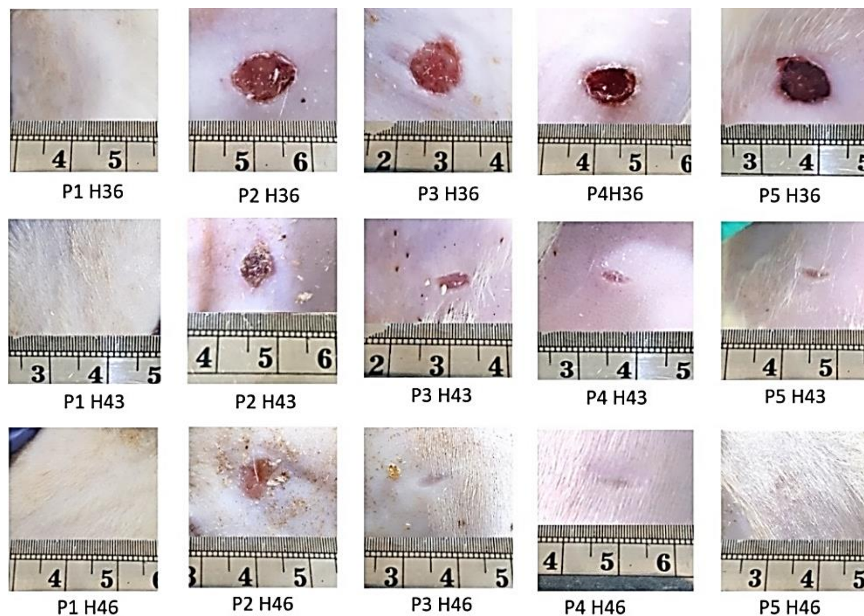

Figure 5. Macroscopic hyperglycemic wounds

Histopathological Results of HE Staining

The readings of histopathological preparations with hematoxylin-eosin (HE) staining showed that the STZ-induced rat group of 65 mg / kgBW had thickened in the epithelium, necrosis in epithelial cells and inflammation in immune cells compared to the group of healthy mice. The results of the analysis of HE histopathological preparations are shown in Figure 6.

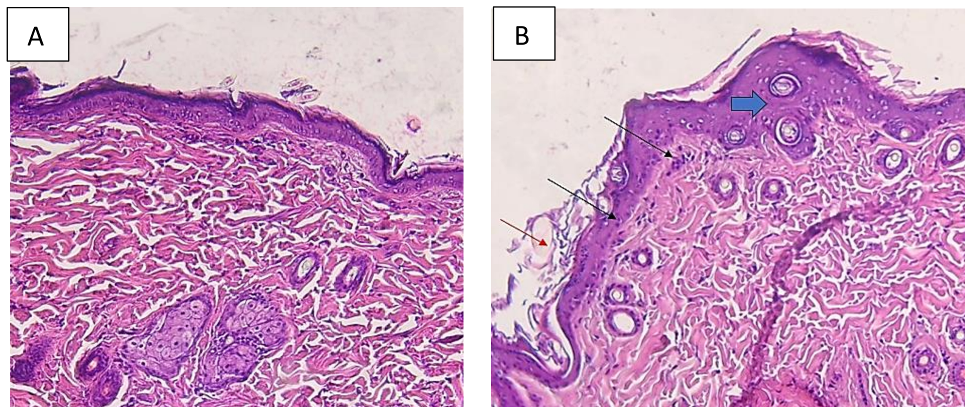

Figure 6. HE Staining Histopathology

Information

A = Group of healthy mice

B = Group of hyperglycemic rats

HE staining in the hyperglycemic group showed thickening of the epithelium (blue arrow), inflammation of immune cells (black arrow), and necrosis of epithelial cells (red arrow).

Effects of MSC Secretome Hypoxia Administration on PDGF Gene Expression

In this study, it was found that MSC secretome hypoxia was able to increase PDGF gene expression in hyperglycemic wound model mice. The results of PDGF gene expression analysis using the RTq-PCR method using the Illumina Eco Real-Time PCR tool are described in Table 2.

Table 2. Mean expression, normality and homogeneity of PDGF between treatment groups

Group	Base gel (P2)	Gentamicin (P3)	Sec 20 µL (P4)	Sec 40 µL (P5)	p value
Rat 1	0,35	0,18	0,66	0,79	
Rat 2	0,25	0,27	0,34	0,27	
Rat 3	0,40	0,12	0,26	0,77	
Rat 4	0,30	0,21	0,66	0,69	
Rat 5	0,25	0,42	0,34	0,35	
Rat 6	0,43	0,13	0,26	0,77	
Mean	0,33	0,22	0,42	0,61	
SD	0,08	0,11	0,19	0,23	
Shapiro wilk	0,42*	0,29*	0,03	0,03	
Levene test	0,007				
Kruskal Wallis	*0,018				

Information:

*Saphiro Wilk test (p > 0.05 = normal)

* Levene’s Test (p > 0,05 = homogenous)

Kruskal Wallis (p<0.05) = significant difference)

Based on Table 2 above, the average results of PDGF gene expression in the base gel group (P2) were 0.33 ± 0.08, the mean gentamicin group (P3) 0.22 ± 0.11, the mean secretome group 20 µL (P4) 0.42 ± 0.19 and the mean secretome group 40 µL (P5) 0.61 ± 0.23 (Figure 6). The average results of PDGF gene expression were normally distributed with Shapiro Wilk test values in the P2=0.42 and P3=0.29 (p>0.05 groups), while normal undistributed data were obtained in the P4=0.03 and P5=0.03 groups with normal values of p>0.05 and inhomogeneous data variants with Levene's Test test results with p=0.007 values with homogeneous values (p>0.05). It was concluded that the data resulting from the expression of PDGF gene groups P4 and P5 were not normally distributed and were not homogeneous.

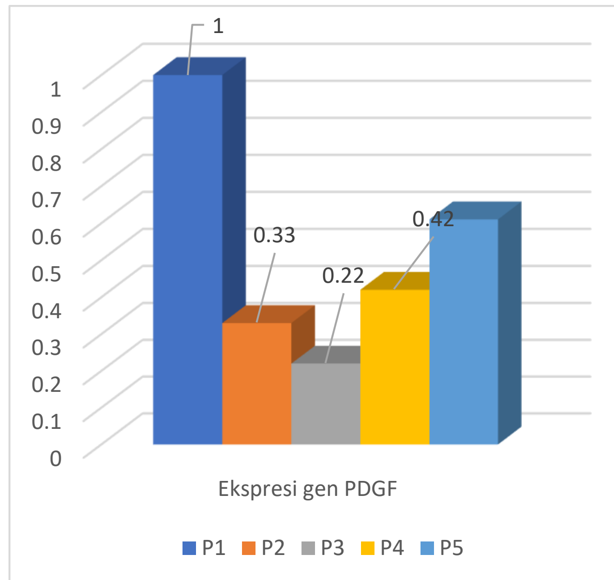

Figure 7. Average PDGF gene expression between treatment groups

Non-parametric statistical analysis using the Kruskal-Wallis test obtained results of 0.018 ($p < 0.05$), it was concluded that there were significant differences between treatment groups on PDGF expression.

Effects of MSC Secretome Hypoxia Administration on IL-1b Gene Expression

The results showed that MSC secretome hypoxia was able to reduce IL-1b gene expression in hyperglycemic wound model mice. The results of IL-1b gene expression analysis using the RTq-PCR method using Illumina's Eco Real-Time PCR tool are described in Table 3.

Table 3. Mean expression, normality, and homogeneity of IL-1b gene expression between treatment groups

Group	Base gel (P2)	Gentamicin (P3)	Sec 20 µL (P4)	Sec 40 µL (P5)	p value
Rat 1	2,14	0,26	0,25	0,34	
Rat 2	1,72	0,41	0,20	0,29	
Rat 3	3,66	0,55	0,26	0,18	
Rat 4	1,39	0,23	0,25	0,10	
Rat 5	2,14	0,41	0,20	0,14	
Rat 6	3,92	0,51	0,26	0,30	
Mean	2,49	0,39	0,24	0,22	
SD	1,04	0,13	0,03	0,09	
Shapiro wilk	0,20*	0,47*	0,17	0,44*	
Levene test	0,000				
Kruskal Wallis	*0,001				
Mann Whitney	*0,004				

Information:

*Saphiro Wilk test (p > 0.05 = normal)

** Levene's Test (p > 0,05 = homogenous)

*Kruskal Wallis (p<0.05) = significant difference)
*Mann Whitney (p<0,05) = different meaning)

Based on Table 3, the average IL-1b gene expression results in the base gel group (P2) were 2.49 ± 1.04, the average gentamicin group (P3) was 0.39 ± 0.13, the average secretome group was 20 µL (P4) was 0.24 ± 0.03 and the average secretome group was 40 µL (P5) was 0.22 ± 0.09.

IL-1b gene expression was highest in the base gel (P2) group and lowest in the 40 µL secretome group, showing that the 40 µL secretome was effective in reducing IL-1b gene expression in mice with hyperglycemic wound models.

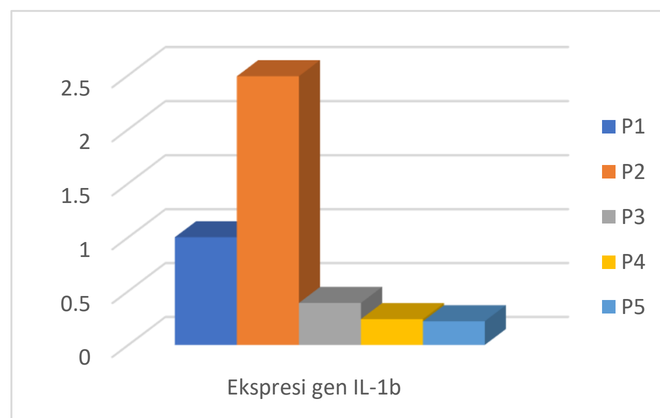

Figure 8. Mean expression, gen IL-1b gene expression between treatment groups

IL-1b gene expression data were normally distributed with Shapiro Wilk test values in the P2=0.20, P3=0.47, and P5=0.44 groups, while in the P4 group, the data were not normally distributed with values of p=0.03 (p > 0.05) and had inhomogeneous data variants with Levene's Test test results with p = 0.000 (p>0.05). It was concluded that the distribution and variant of IL-1b gene expression data are not normally distributed and are not homogeneous. The Kruskal-Wallis non-parametric test obtained results p= 0.001 (p < 0.05), followed by the Mann-Whitney test obtained results of 0.004 (p < 0.05) so that it was concluded that there were significant differences between treatment groups of IL-1b gene expression in mice with hyperglycemic wound models.

IV. DISCUSSION

Inflammation is a local response to various inflammatory cells to eliminate, cleanse, build, and maintain the integrity of the tissue homeostasis system. The inflammatory process is initiated by inflammatory cells due to the stimulation of danger signal molecules produced when pathogenic bacteria damage certain tissues or substances.[63] Impaired wound healing due to hyperglycemia alters endothelial cell activity and causes vascular dysfunction in wounds, increasing the risk of infection up to amputation. Changes in a number of endogenous factors, including decreased growth factor production, angiogenic response mediators, macrophage activity, collagen synthesis, keratinocyte migration, and fibroblast proliferation lead to delayed wound healing.[59] Ormazabal et al, 2022 study using MSC-EC hypoxic secretome or specific protein combination enriched in MSC-EC hypoxic secretome improves the wound healing process in hyperglycemic conditions.[57] The group that received secretome treatment showed rapid wound healing and less scarring. In addition, further research using the human fibroblast secretome serves as a human protein control. When wounds are treated with stem cell secretion, epidermal and dermal thickness increases markedly faster.[17]

MSCs actively contribute to angiogenesis through direct differentiation, interaction of cell contact with endothelial cell derivatives, and paracrine release of pro-angiogenic molecules. Paracrine factor can increase the blood supply of damaged tissues through the activation and recruitment of stem cells and progenitor cells.[66]

The results of the PDGF gene expression study in the base gel group (P2) were 0.33 ± 0.08, showed an increase in the average gentamicin group (P3) 0.22 ± 0.11, the average secretome group 20 µL (P4) 0.42 ± 0.19 showed an increase from the P3 group and the average secretome group of 40 µL (P5) showed the highest increase of 0.61 ± 0.23, It can be concluded that the administration of MSC secretome hypoxia gel a dose of 40 µL/rat was significant for increased expression of the PDGF gene in male white rats of the Wistar strain model of hyperglycemic wounds. In line with research by Chen et al, 2020 routine use can increase the production of antioxidant enzymes induced by nuclear factor erythroid 2-related factor 2 (NRF2), inhibit the expression of matrix metalloproteinases (MMPs) regulated by NF-κB, and decrease the expression of vascular endothelial growth factor (VEGF). It also promotes the expression of the associated neurogenic protein (UCH-L1) reduces oxidative stress and

inflammatory response in hyperglycemic rats, promotes wound healing, and reduces the risk of ulcers.[1] PDGF acts as one of the growth and division factors, especially during the angiogenesis phase and the formation of blood vessels from the previous vascular network. MSC mechanism in the wound area through the process of differentiation, and vascularization by involving the release of PDGF.[61]

Another study by Das et al, 2016. reported a significant increase in wound healing activity with co-administration of PDGF-BB with syndecan-4 proteoliposomes (S4PL). significant translational potential in enhancing the effects of PDGF-BB for diabetic wound healing. The addition of syndecan-4 co-treatment may improve keratinocyte migration and wound closure in the context of diabetes S4PL has an immunomodulatory effect on wound macrophages, reduces inflammation, and improves phenotypic markers of M2 macrophages.[51]

The results of the IL-1b gene expression in the base gel group (P2) of 2.49 ± 1.04, experienced an average decrease in the gentamicin group (P3) of 0.39 ± 0.13, the average in the secretome group of 20 µL (P4) decreased from the P3 group to 0.24 ± 0.03 and the lowest average expression of the IL-1b gene in the secretome group of 40 µL (P5) of 0.22 ± 0.09. Showed that administration of MSC secretome hypoxia gel dose 40 µL/rat effectively decreased IL-1b gene expression in mice with hyperglycemic wound models. In line with research by Shiddiqi et al, 2017. IL-1 and Caspase-1 expression can be lowered in mice with pristane-induced lupus with MSC secretome administration. This is indicated by the fact that IL-1β and Caspase-1 are expressed to a lesser extent in the pristane + secretome group compared to the pristane group.[67]

The decrease in caspase-1 and IL-1b expression is caused by inhibition of NLRP3 inflammasome activation so that caspase-1 activation does not occur which causes IL-1b not to form. Inhibition of NLRP3 inflammasome activation is caused because the MSC secretome contains stanniocalcin-1 (STC-1) which also plays a role in inhibiting ROS production.[68] Administration of MSC secretome inhibits NLRP3 inflammasome by lowering ROS levels in mitochondria. ROS plays an important role in activating the NLRP3 inflammasome. NLRP3 inflammasome activates caspase-1 which converts pro IL-1b into active IL-1b which plays a role in inflammatory mechanisms.[67] Peripheral blood angiocrine MSCs can stimulate endothelial functional characteristics by activating the VEGF-A signaling pathway through a number of factors, including endothelin-1, IL-8, platelet-derived growth factor-AA (PDGF-AA), and IGF-2. MSC hypoxia expresses much higher levels of Heat Shock Protein (HSP) molecules than normoxic, capable of folding proteins that lead to optimal intracellular protein function, such as VEGF and PDGF. Hypoxic MSCs are more effective in regenerating damaged tissue than normoxic MSCs.[66]

V. CONCLUSION

MSC secretome hypoxia gel administration significantly affected PDGF and IL-1b gene expression in male white rats of Wistar strain model hyperglycemic wound.

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REFERENCES

1) Chen LY, Huang CN, Liao CK, et al. Effects of rutin on wound healing in hyperglycemic rats. Antioxidants. 2020;9(11):1-13. doi:10.3390/antiox9111122
2) Rahmadani S. Aplikasi Topikal Ekstrak Etanol Daun Sirsak (Annona Muricata L.) Pada Luka Tikus Hiperglikemia Setelah Diberi Pakan Lemak Tinggi. http://etd.repository.ugm.ac.id/
3) Wang L, Wang HL, Liu TT, Lan HY. TGF-beta as a master regulator of diabetic nephropathy. Int J Mol Sci. 2021;22(15). doi:10.3390/ijms22157881
4) Mirza RE, Fang MM, Ennis WJ, Kohl TJ. Blocking interleukin-1β induces a healing-associated wound macrophage phenotype and improves healing in type 2 diabetes. Diabetes. 2013;62(7):2579-2587. doi:10.2337/db12-1450
5) Shen S, Wang F, Fernandez A, Hu W. Role of platelet-derived growth factor in type II diabetes mellitus and its complications. Diab Vasc Dis Res. 2020;17(7). doi:10.1177/1479164120942119
6) Dan P, Diabetes P, Tipe M. Perkumpulan Endokrinologi Indonesia.; 2011.
7) Kuntardjo N, Dharmana E, Chodidjah C, Nasihun TR, Putra A. TNF-α-Activated MSC-CM Topical Gel Effective in Increasing PDGF Level, Fibroblast Density, and Wound Healing Process Compared to Subcutaneous Injection Combination. Majalah Kedokteran Bandung. 2019;51(1):1-6. doi:10.15395/mkb.v51n1.1479
8) HS Z, Putra A. Peran Mesenchymal Stem Cells dalam Regulasi PDGF dan Sel Islet pada Diabetes. Jurnal Kedokteran Brawijaya. 2018;30(2):98-102. doi:10.21776/ub.jkb.2018.030.02.4

9) Badr G, Badr BM, Mahmoud MH, Mohany M, Rabah DM, Garraud O. Treatment of diabetic mice with undenatured whey protein accelerates the wound healing process by enhancing the expression of MIP-1α, MIP-2, KC, CX3CL1 and TGF-β in wounded tissue. BMC Immunol. 2012;13. doi:10.1186/1471-2172-13-32
10) Kulkarni M, O'Loughlin A, Vazquez R, et al. Use of a fibrin-based system for enhancing angiogenesis and modulating inflammation in the treatment of hyperglycemic wounds. Biomaterials. 2014;35(6):2001-2010. doi:10.1016/j.biomaterials.2013.11.003
11) Regmi S, Pathak S, Kim JO, Yong CS, Jeong JH. Mesenchymal stem cell therapy for the treatment of inflammatory diseases: Challenges, opportunities, and future perspectives. Eur J Cell Biol. 2019;98(5-8). doi:10.1016/j.ejcb.2019.04.002
12) Park SR, Kim JW, Jun HS, Roh JY, Lee HY, Hong IS. Stem Cell Secretome and Its Effect on Cellular Mechanisms Relevant to Wound Healing. Molecular Therapy. 2018;26(2):606-617. doi:10.1016/j.ymthe.2017.09.023
13) Vaidyanathan L. Growth factors in wound healing ⇓ a review. Biomedical and Pharmacology Journal. 2021;14(3):1469-1480. doi:10.13005/bpj/2249
14) Jung H, Son GM, Lee JJ, Park HS. Therapeutic effects of tonsil-derived mesenchymal stem cells in an atopic dermatitis mouse model. In Vivo (Brooklyn). 2021;35(2):845-857. doi:10.21873/INVIVO.12325
15) Bormann D, Gugerell A, Ankersmit HJ, Mildner M. Therapeutic Application of Cell Secretomes in Cutaneous Wound Healing. Journal of Investigative Dermatology. 2023;143(6):893-912. doi:10.1016/j.jid.2023.02.019
16) Karuniawan A, Andrie M, Riza H, Studi Farmasi P, Kedokteran F, Tanjungpura Pontianak U. Uji Efek Penyembuhan Luka Sayat Salep Ekstrak Ikan Toman (Channa Micropeltes) Secara Topikal Pada Tikus Yang Diinduksi Streptozotocin.; 2016.
17) Park SR, Kim JW, Jun HS, Roh JY, Lee HY, Hong IS. Stem Cell Secretome and Its Effect on Cellular Mechanisms Relevant to Wound Healing. Molecular Therapy. 2018;26(2):606-617. doi:10.1016/j.ymthe.2017.09.023
18) Ahmadi H, Amini A, Fadaei Fathabady F, et al. Transplantation of photobiomodulation-preconditioned diabetic stem cells accelerates ischemic wound healing in diabetic rats. Stem Cell Res Ther. 2020;11(1). doi:10.1186/s13287-020-01967-2
19) Hu W, Zhang Y, Wang L, et al. Bone morphogenic protein 4-smad-induced upregulation of platelet-derived growth Factor AA impairs endothelial function. Arterioscler Thromb Vasc Biol. 2016;36(3):553-560. doi:10.1161/ATVBAHA.115.306302
20) Mulholland EJ. Electrospun Biomaterials in the Treatment and Prevention of Scars in Skin Wound Healing. Front Bioeng Biotechnol. 2020;8. doi:10.3389/fbioe.2020.00481
21) Manzat Saplacan RM, Balacescu L, Gherman C, et al. The Role of PDGFs and PDGFRs in Colorectal Cancer. Mediators Inflamm. 2017;2017. doi:10.1155/2017/4708076
22) Farooqi AA, Siddik ZH. Platelet-derived growth factor (PDGF) signalling in cancer: Rapidly emerging signalling landscape. Cell Biochem Funct. 2015;33(5):257-265. doi:10.1002/cbf.3120
23) Folestad E, Kunath A, Wågsäter D. PDGF-C and PDGF-D signaling in vascular diseases and animal models. Mol Aspects Med. 2018;62:1-11. doi:10.1016/j.mam.2018.01.005
24) Tan JL, Lash B, Karami R, et al. Restoration of the healing microenvironment in diabetic wounds with matrix-binding IL-1 receptor antagonist. Commun Biol. 2021;4(1). doi:10.1038/s42003-021-01913-9
25) Larouche J, Sheoran S, Maruyama K, Martino MM. Immune regulation of skin wound healing: Mechanisms and novel therapeutic targets. Adv Wound Care (New Rochelle). 2018;7(7):209-231. doi:10.1089/wound.2017.0761
26) Rea IM, Gibson DS, McGilligan V, McNerlan SE, Denis Alexander H, Ross OA. Age and age-related diseases: Role of inflammation triggers and cytokines. Front Immunol. 2018;9(APR). doi:10.3389/fimmu.2018.00586
27) Zhang X, Dai J, Li L, Chen H, Chai Y. NLRP3 Inflammasome Expression and Signaling in Human Diabetic Wounds and in High Glucose Induced Macrophages. J Diabetes Res. 2017;2017. doi:10.1155/2017/5281358
28) Dai J, Shen J, Chai Y, Chen H. IL-1 β Impaired Diabetic Wound Healing by Regulating MMP-2 and MMP-9 through the p38 Pathway. Mediators Inflamm. 2021;2021. doi:10.1155/2021/6645766
29) Mirza RE, Fang MM, Weinheimer-Haus EM, Ennis WJ, Koh TJ. Sustained inflammasome activity in macrophages impairs wound healing in type 2 diabetic humans and mice. Diabetes. 2014;63(3):1103-1114. doi:10.2337/db13-0927
30) Liu Y, Liu Y, Deng J, Li W, Nie X. Fibroblast Growth Factor in Diabetic Foot Ulcer: Progress and Therapeutic Prospects. Front Endocrinol (Lausanne). 2021;12. doi:10.3389/fendo.2021.744868
31) Boniakowski AE, Kimball AS, Joshi A, et al. Murine macrophage chemokine receptor CCR2 plays a crucial role in macrophage recruitment and regulated inflammation in wound healing. Eur J Immunol. 2018;48(9):1445-1455. doi:10.1002/eji.201747400
32) Liu D, Yang P, Gao M, et al. NLRP3 activation induced by neutrophil extracellular traps sustains inflammatory response in the diabetic wound. Clin Sci. 2019;133(4):565-582. doi:10.1042/CS20180600

33) Putra A, Pertiwi D, Milla MN, et al. Hypoxia-preconditioned MSCs have superior effect in ameliorating renal function on acute renal failure animal model. Open Access Maced J Med Sci. 2019;7(3):305-310. doi:10.3889/oamjms.2019.049
34) Vasanthan J, Gurusamy N, Rajasingh S, et al. Role of human mesenchymal stem cells in regenerative therapy. Cells. 2021;10(1):1-14. doi:10.3390/cells10010054
35) Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: Past, present, and future. Stem Cell Res Ther. 2019;10(1). doi:10.1186/s13287-019-1165-5
36) Putra A, Ridwan FB, Putridewi AI, et al. The role of tnf-α induced mscs on suppressive inflammation by increasing tgf-β and il-10. Open Access Maced J Med Sci. 2018;6(10):1779-1783. doi:10.3889/oamjms.2018.404
37) Ullah M, Liu DD, Thakor AS. Mesenchymal Stromal Cell Homing: Mechanisms and Strategies for Improvement. doi:10.1016/j.isci
38) Ghaneialvar H, Soltani L, Rahmani HR, Lotfi AS, Soleimani M. Characterization and Classification of Mesenchymal Stem Cells in Several Species Using Surface Markers for Cell Therapy Purposes. Indian Journal of Clinical Biochemistry. 2018;33(1):46-52. doi:10.1007/s12291-017-0641-x
39) Lotfinia M, Lak S, Ghahhari NM, et al. Full Length Iranian Hypoxia Pre-Conditioned Embryonic Mesenchymal Stem Cell Secretome Reduces IL-10 Production by Peripheral Blood Mononuclear Cells. Biomed J. 2017;21(1):24-31. doi:10.6091/.21.1.24
40) Madrigal M, Rao KS, Riordan NH. A review of therapeutic effects of mesenchymal stem cell secretions and induction of secretory modification by different culture methods. J Transl Med. 2014;12(1). doi:10.1186/s12967-014-0260-8
41) Drawina P, Putra A, Nasihun T, Prajoko YW, Dirja BT, Amalina ND. Increased serial levels of platelet-derived growth factor using hypoxic mesenchymal stem cell-conditioned medium to promote closure acceleration in a full-thickness wound. Indones J Biotechnol. 2022;27(1):36-42. doi:10.22146/ijbiotech.64021
42) Meng X, Grötsch B, Luo Y, et al. Hypoxia-inducible factor-1α is a critical transcription factor for IL-10-producing B cells in autoimmune disease. Nat Commun. 2018;9(1). doi:10.1038/s41467-017-02683-x
43) Syafril S. Pathophysiology diabetic foot ulcer. In: IOP Conference Series: Earth and Environmental Science. Vol 125. Institute of Physics Publishing; 2018. doi:10.1088/1755-1315/125/1/012161
44) Alavi A, Sibbald RG, Mayer D, et al. Diabetic foot ulcers: Part I. Pathophysiology and prevention. J Am Acad Dermatol. 2014;70(1):1.e1-1.e18. doi:10.1016/j.jaad.2013.06.055
45) Aumiller WD, Dollahite HA. Pathogenesis and management of diabetic foot ulcers. J Am Acad Physician Assist. 2015;28(5):28-34. doi:10.1097/01.JAA.0000464276.44117.b1
46) Noor S, Zubair M, Ahmad J. Diabetic foot ulcer - A review on pathophysiology, classification and microbial etiology. Diabetes and Metabolic Syndrome: Clinical Research and Reviews. 2015;9(3):192-199. doi:10.1016/j.dsx.2015.04.007
47) Chantelau EA. Nociception at the diabetic foot, an uncharted territory. World J Diabetes. 2015;6(3):391. doi:10.4239/wjd.v6.i3.391
48) Jhamb S, Vangaveti VN, Malabu UH. Genetic and molecular basis of diabetic foot ulcers: Clinical review. J Tissue Viability. 2016;25(4):229-236. doi:10.1016/j.jtv.2016.06.005
49) Wang CJ, Ko JY, Kuo YR, Yang YJ. Molecular changes in diabetic foot ulcers. Diabetes Res Clin Pract. 2011;94(1):105-110. doi:10.1016/j.diabres.2011.06.016
50) Blakytny R, Jude EB. Altered molecular mechanisms of diabetic foot ulcers. International Journal of Lower Extremity Wounds. 2009;8(2):95-104. doi:10.1177/1534734609337151
51) Das S, Majid M, Baker AB. Syndecan-4 enhances PDGF-BB activity in diabetic wound healing. Acta Biomater. 2016;42:56-65. doi:10.1016/j.actbio.2016.07.001
52) Wang L, Wang F, Zhao L, et al. Mesenchymal stem cells coated by the extracellular matrix promote wound healing in diabetic rats. Stem Cells Int. 2019;2019. doi:10.1155/2019/9564869
53) Okonkwo UA, Dipietro LA. Diabetes and wound angiogenesis. Int J Mol Sci. 2017;18(7). doi:10.3390/ijms18071419
54) Kartika RW, Alwi I, Suyatna FD, et al. The Role Of Vegf, Pdgf And Il-6 On Diabetic Foot Ulcer After Platelet Rich Fibrin Þ Hyaluronic Therapy. Heliyon. 2021;7(9). doi:10.1016/j.heliyon.2021.e07934
55) MacLeod AS, Mansbridge JN. The Innate Immune System in Acute and Chronic Wounds. Adv Wound Care (New Rochelle). 2016;5(2):65-78. doi:10.1089/wound.2014.0608
56) Raziyeva K, Kim Y, Zharkinbekov Z, Kassymbek K, Jimi S, Saparov A. Immunology of acute and chronic wound healing. Biomolecules. 2021;11(5). doi:10.3390/biom11050700

57) Ormazabal V, Nova-Lampeti E, Rojas D, et al. Secretome from Human Mesenchymal Stem Cells-Derived Endothelial Cells Promotes Wound Healing in a Type-2 Diabetes Mouse Model. Int J Mol Sci. 2022;23(2). doi:10.3390/ijms23020941
58) Federer WT. Experimental Design Theory And Application, Third Edition,. Oxford and IBH Publishing Co, New Delhi Bombay Calcuta. Published online 1977.
59) Beserra FP, Vieira AJ, Gushiken LFS, et al. Lupeol, a dietary triterpene, enhances wound healing in streptozotocin-induced hyperglycemic rats with modulatory effects on inflammation, oxidative stress, and angiogenesis. Oxid Med Cell Longev. 2019;2019. doi:10.1155/2019/3182627
60) Widyaningsih W. Pengaruh Pemberian Sekretom Sel Punca Mesenkimal Hipoksia Terhadap Kadar C-Peptide, IL-6, Dan Polarisasi Makrofag Tipe-2 (Studi Eksperimental in Vivo Pada Tikus Obesitas Diabetes Melitus Tipe 2) (Doctoral Dissertation, Universitas Islam Sultan Agung (Indonesia)).; 2022.
61) HS Z, Putra A. Peran Mesenchymal Stem Cells dalam Regulasi PDGF dan Sel Islet pada Diabetes. Jurnal Kedokteran Brawijaya. 2018;30(2):98-102. doi:10.21776/ub.jkb.2018.030.02.4
62) Muhar AM, Putra A, Warli SM, Munir D. Hypoxia-mesenchymal stem cells inhibit intra-peritoneal adhesions formation by upregulation of the il-10 expression. Open Access Maced J Med Sci. 2019;7(23):3937-3943. doi:10.3889/oamjms.2019.713
63) Putra A. Basic Molecular Stem Cell Semarang: Unisulla Press.; 2019.
64) Matluobi D, Araghi A, Maragheh BFA, et al. Carvacrol promotes angiogenic paracrine potential and endothelial differentiation of human mesenchymal stem cells at low concentrations. Microvasc Res. 2018;115:20-27. doi:10.1016/j.mvr.2017.08.003
65) Kehl D, Generali M, Mallone A, et al. Proteomic analysis of human mesenchymal stromal cell secretomes: a systematic comparison of the angiogenic potential. NPJ Regen Med. 2019;4(1). doi:10.1038/s41536-019-0070-y

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