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Adipose Derived Stem Cells Enhanced Diabetic Wound Healing and Improved Ultrastructural Morphology of Skin Tissue Through Upregulating Growth Factors Expression.

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Abstract
Foot ulcers are one of the most common and severe complications of diabetes mellitus, with substantial morbidity and mortality. The study displayed the effect of a dipose-derived stem cells (ADSCs)on wound healing in diabetic rats. Diabetes was induced by streptozotocin (STZ) through a single I.P. injection. A full-thickness wound about 10mm in diameter was formed one week after inducing diabetes. Twenty adult male albino rats were divided into Diabetic rats(diabetic with non-treated skin wounds), and stem cellstreated rats (diabetic wounds were treated with a single intradermal injection of $1X10^6$ ADSCs). Fourteen days after making the wound, samples from the wound treated by stem cells revealed a marked increase in the wound closure percentage compared to the diabetic group. The finding was reinforced by histological analysis and electron microscopic study. Also, the gene expression of epidermal growth factor (EGF) and insulin-like growth factor (IGF) were assessed by qRT-PCR. The treated stem cells showed increased EGF and IGF levels compared to the diabetic group. So, the ADSC scan accelerate diabetic wound healing by increasing the growth factors like EGFand IGF.

Keywords:
Diabetic wound; ADSCs; EGF; IGF; wound healing.
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1. Introduction

Foot ulcers are one of the most common and severe complication of diabetes mellitus with substantial morbidity and mortality[1].

The biological process of wound healing occurs in skin tissue following trauma, burns, or diabetic ulcers. For instance, long-term bedridden individuals with diabetes may have chronic skin lesions that are difficult to cure. As a result, diabetic wound healing is one of the most difficult problems for doctors to solve with significant financial and medical burden for individuals [2].

Oxygen supply to the wound in diabetic patients is inadequate due to vascular dysfunction and neuropathy. In addition to inadequate oxygen supply, there is imbalance between angiogenic factors like transforming growth factor-βand angiostatic factors like angiostatin leading to angiogenic imbalance and increase wound hypoxia[3].

the ability to differentiate into connective tissue lineage cells. Another source of stromal cells that has been identified is adipose-derived mesenchymal stem cells[5].

Bone marrow, periosteum, umbilical cord blood, dermis, infrapatellar fat pad, adipose tissue, synovium and skeletal muscles have all been found to contain mesenchymal stem cells [4]. They are multipotent stem cells with

The application of stem cells in therapy has grown because they are pluripotent, self-renewing and can stimulate the release of regenerative cytokines[6]. Stem cells have been thought to promote wound healing by regulating macrophages, reducing inflammation, secreting VEGF to stimulate angiogenesis, encouraging proliferation and differentiation of fibroblasts and keratinocyte, producing anti-fibrosis cytokines[7].

Treatment of diabetic foot ulcers remain a great challenge. Wound-healing strategies comprise standard therapies and advanced therapies. The standard of care treatment includes wound debridement and glycemic and infection control. However, advanced therapies include hyperbaric oxygen therapy , wound dressings and negative pressure wound therapy[8].

2.Methodology

2.1. Drugs

The drugs were supplied as follow. Citrate buffer (pH 4.5) and Streptozotocin (STZ): (Sigma Chemicals Co., St. Louis, MO, USA).

2.2. Animals

Twenty adult male albino rats with average weight (180- 200 grams) were used in this study. The rats were kept in the animal houseof Faculty of Pharmacy; Kafrelsheikh University. All the procedures of the experiment were done according to guidelines approved by the Animal Use Committee of Kafrelsheikh University.

2.3. Induction of type I diabetes mellitus

Before receivingSTZ injection,the rats were fasted overnight. STZ was given in a dose of 60 mg/kg body weightby intraperitoneal (I.P.) injection. The powder of STZ was dissolved in freshly prepared cold citrate buffer (pH 4.5) and then injected immediately [9].

The rats established decrease in their weight and hyperglycemia, which occurs also in diabetic humans[10,11]. The fasting blood glucose levels (FBG) were measured using a glucometer (Accu-Chek Active, Roche Diagnostics, Germany) three days after STZ injection to make sure that the diabetes was established. The rats with a blood glucose level more than 250 mg/dl were considered diabetic and included in the study. As a continual monitoring, the FBG was measured again on days7 and 14[12].

2.4. Formation of full thickness wound

The wounds were created after confirming that diabetes had been induced. It was formed one week after the diabetes was established. The back hair of the rats wasshaved after giving ketamine hydrochloride I.P. injections (90 mg/kg/rat) [13]. Afterwards, full thickness circular wounds about 10mm in diameter were made in the rats upper back with a scalpel blade [11,14].

2.5. Preparation of ADSCs isolated from rats

The isolation and expansion of ADSCs were madein the Biochemistry Department, Faculty of Medicine, Cairo University

2.6. Groups and Treatment

The animals were divided into two groups (n=10 rats/ group).

- Diabetic group: diabetic rats with non-treated skin wounds.
- Stem cells group: diabetic wounds wereinjected intradermally with $1X10^6$ ADSCs dissolved in 1 ml phosphate-buffered saline (PBS). They were injected once around the wound margins [15].

2.7. Assessment of wounds closure

The wounds were photographed with digital camera to measure diameters of wounds [16]. The wound closure percentage (%) was evaluated by the Wilson's formula mentioned as % of wounds closure = [(Area on 0 day − Area of X days)/Area on 0 day] × 100% [13].

2.8. Histological Analysis

Specimens from the wound tissue were taken with about 1 cm normal skin margin. Thespecimens were placed immediately in 10% buffered formalin for 1 day.Paraffin blocks were processed and sections of 5µm thick were subjected to Hematoxylin and Eosin (H&E) stain for recognition of histological changes and Masson's trichrome to detect collagen [17].

2.9. Electron microscopic study

Biopsies from the wound were taken and cut into small pieces. They were put and fixed in buffered glutaraldehyde, processed and finally examined using a transmission electron microscope (TEM) in the E.M. Center, Faculty of Medicine, Tanta University [18].

2. 10. Quantitative Real time Polymerase Chain Reaction (qRT -PCR)

Quantitative determination of the levels of epidermal growth factor (EGF) and insulin like growth factor (IGF) were performed using quantitative qRT -PCR technique. Samples from wounded tissue were taken as soon as the sacrifice was made (on day14) and were kept in Qiazol Lysis Reagent (Cat. #: 79306, Qiagen, Cairo, Egypt) and

2. 10.2 Reverse transcription of extracted RNA followed by PCR in one step

Reverse transcription of extracted RNA was performed using the Super Script IV One-Step RT-PCR kit (Cat# 12594100, Thermo Fisher Scientific, Waltham, MA, USA) and followed by PCR in a single step.

stored at -80 ◦C until the time of analysis[19].

2. 10.1 RNA extraction

Following the manufacturer's instructions, total RNA was extracted from all enrolled samples using Direct-zol RNA Miniprep Plus (Cat. # R2072, ZYMO RESEARCH CORP. USA). The Beckman dual spectrophotometer USA was then used to determine the quantity and quality of the extracted RNA.

Table 1. Primers sequence of all studied genes:

	Forward sequence	Reverse sequence
EGF	TGGTGCCGGGTCTGATGATG	GCAATGCGGTTCTGATACTG
IGF	TAGGTGGTTGATGAATGGT	GAAAGGGCAGGGCTAAT
GAPDH	CACCCTGTTGCTGTAGCCATATTC	GACATCAAGAAGGTGGTGAAGCAG

±15.20 mg/dl) in the stem cells group. On day fourteen, the mean serum glucose levels were (555.3 ±29.41 mg/dl) in the diabetic group and (562.5±30.39 mg/dl)in the stem cells group. There was no significant difference in serum glucose levels between diabetic and stem cells groups in the two durations. The animals developed weight loss and polyuriaas diabetic people. The animals' weight loss is due to severe dehydration and inadequate insulin after STZ injection, which hinders the body's cells' capability to absorb blood glucose. The animals death occurs due to toxic effects of STZ on many body organs and not due to complications of diabetic mellitus [10,20].

3.2. Wound closure evaluation

Examination of the wound on day 14 in the different groups, the diabetic group displayed a noticeable delay of wound healing with significant reduction in the wound closure % as compared to stem cells group. On the other hand, the diabetic skin wounds treated with stem cells revealed a substantial increase in the wound closure % as compared to diabetic group. Consistent with this, the study performed by [21]stated that, compared to untreated controls, ADSCs-treated wounds showed marked reductions in the ulcers' diameter, depth and associated pain with no recorded adverse events.

2.10.3. Calculation of Relative Quantification (RQ) (relative expression):

After the RT-PCR run, the data were expressed in Cycle threshold (Ct). The PCR data sheet includes Ct values of assessed gene (***EGFand IGF)***versus the corresponding the house keeping gene (*GAPDH*) (Table 1). In order to measure the gene expression of certain gene, a control sample should be used. The RQ of each target gene is quantified and normalized to housekeeping gene according to the calculation of delta-delta Ct (ΔΔCt). We calculated the RQ of each gene by taking $2^{-\Delta\Delta Ct}$.

2.11. Statistical Analysis

All results were xpressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA). A P-value less than 0.05 was considered to be statistically significant.

3. Results and discussion

3.1.Laboratory results

The rats injected with STZ showed hyperglycemia and maintained it throughout the study. Seven days after STZ injection, the mean serum glucose level was (420.8 ±16.13mg/dl) in the diabetic group and (417.2

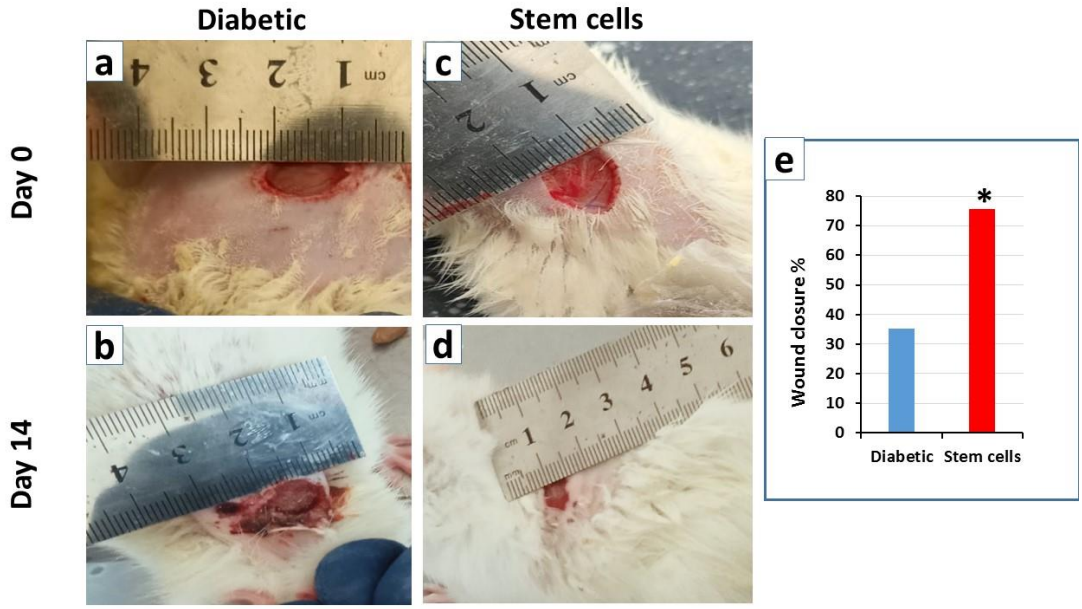

Fig. 1. (a,b,c,d) Displaying wound areas in diabetic and stem cells groups from days 0 and 14 post wound induction. (e) Histogram of wound closure % in the different groups from day 14 after wound induction, *: significant compared to diabetic group (p value ≤ 0.05).

keratinocytes, which results in insufficient re-epithelialization and delayed healing[22]. On examination of the stem cells group, the wound area was contracted due to mild inflammatory cells and the dermis contained more collagen and dilated blood vessels. Stem cells can migrate to an inflammatory site and encourage the proliferation of resident progenitor cells [23].

3.3. Hematoxylin and Eosin (H&E) results

Hematoxylin and Eosin stained sections from diabetic group displayed disorganized granulation tissue with numerous inflammatory cells (Fig. 2). Diabetic wounds are characterized by high levels of reactive oxygen species (ROS). These ROS can cause DNA damage and inactivation of enzymes that forage free radicals [9].Hyperglycemia hampers the migration and proliferation of

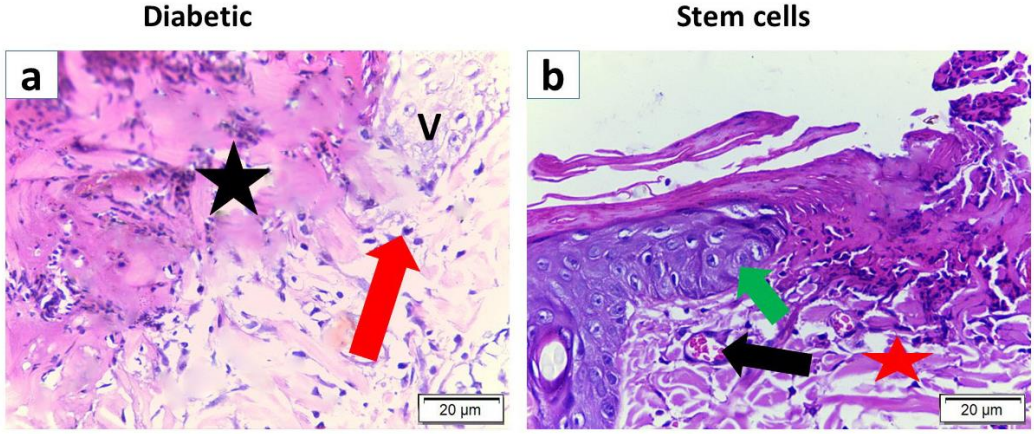

Fig. 2. H&E stained skin sections from diabetic control and stem cells groups on day 14. (a) Diabetic rat showing granulation tissue filling the wound gap with numerous inflammatory cells (red arrow), covered with scab with numerous inflammatory cells (black star)and cytoplasmic vacuolations in the epithelium in the edge of the wound (V), (X:400, scale bar 20 µm). (b)Stem cellstreated rat showing normal epithelialization (green arrow) in the wound edge, organized granulation tissue with dilated blood vessels (black arrow) and more collagen fibers (red star) (X:400, scale bar 20 µm).

collagen deposition as compared stem cells group(Fig. 3). Consistent with this finding[9,11]observed fine irregularly distributed collagen. On the other hand, the stem cells group revealed marked increase in the mean area % ofcollagen deposition as

3.4. Masson's trichrome results

Masson's trichrome stained skin sections from diabetic rats showed that the wound beds after 14 days was filled with fine, transversally arranged collagen fibers with significant decrease in the mean area % of

augmenting fibroblastic proliferation and synthesis [24].

compared to diabetic group. The wound bed was filled with dense collagen bundles. ADSCs can produce large number of growth factors

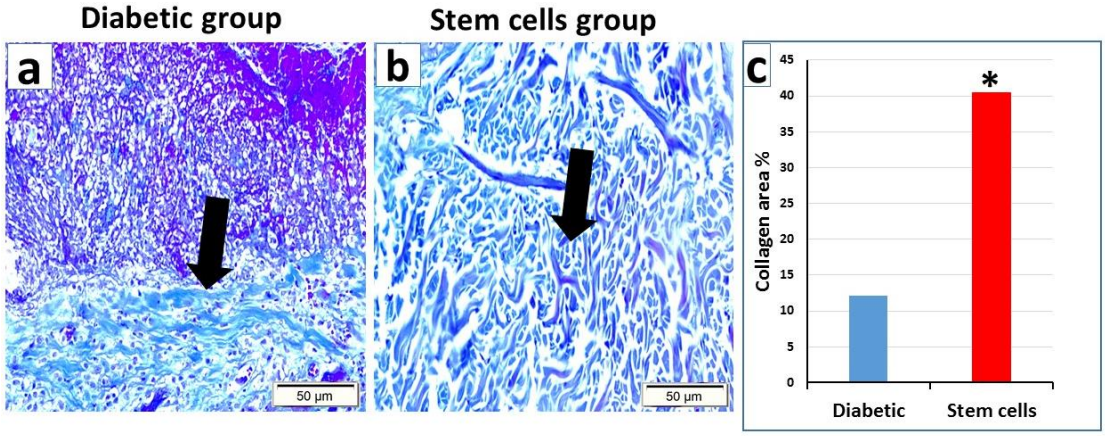

Fig. 3. (a,b) Masson trichrome stained skin Images from diabetic and stem cells groups on day 14. (a) Diabetic rat showing the wounds bed is filled with thin, faint and transversallyarranged collagen fibers (arrow). (b) Diabetic rats treated with stem cellsshowing the wound beds filled with dense irregular collagen bundles (arrow), (X:200, scale bar 50 µm). (c) statistical analysis of collagen area % of the diabetic and stem cells groups, *: Significant compared to diabetic group (p value ≤ 0.05).

decrease in phagocytic functions of macrophages and neutrophils cells[25].

Ultrathin sections from the stem cells group (Fig. 4)displayedstratum spinosum cell with euchromatic nucleus, normal intercellular space with regular desmosomesand to no filament bundles. Also, active fibroblast with euchromatic nucleus and more collagen bundles were seen in the dermis. ADSCs enhance keratinocyte migration and proliferation [13,26]

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3.5. EM results

In the present study, ultrathin sections from the diabetic group(Fig. 4) showed stratum spinosum cell with widening of intercellular space irregular desmosomes, fragmented to no filament bundles. Also, fibroblast with heterochromatic nucleus and scarce collagen fibers were seen in the dermis. Impaired diabetic wound healing is due to prolonged inflammatory process which occurs due to

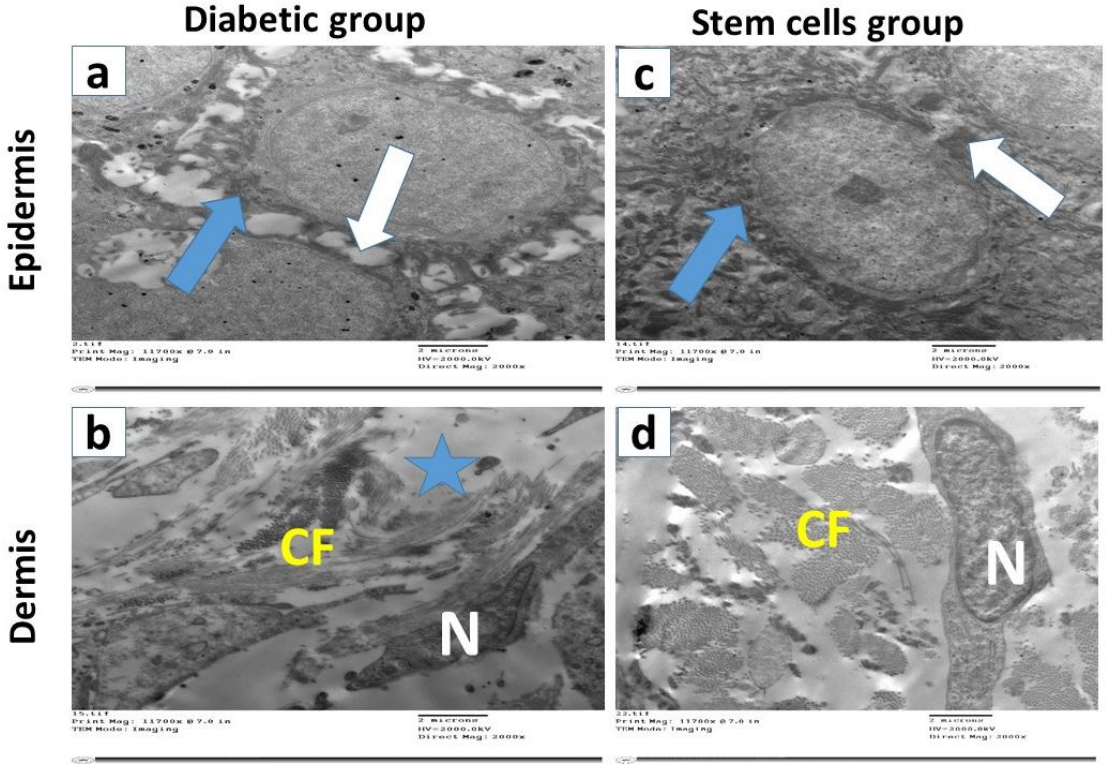

Fig. 4. Ultrathin images from the wound tissue in diabetic and stem cells groups on day 14 post wound induction. (a, b) Diabetic rat showing stratum spinosum cell with widening of intercellular space

(white arrow), fragmented to no filament bundles (blue arrow). Also, fibroblast with heterochromatic nucleus (N) and scarce collagen fibers (CF) and empty spaces (star) are seen in the dermis. (c,d) stem cells treated rat showing stratum spinosum cell with euchromatic nucleus, normal intercellular space with regular desmosomes (white arrow) and tonofilament bundles (blue arrow). Also, active fibroblast with euchromatic nucleus (N) and more collagen bundles (CF) are seen in the dermis.

encourageskeratinocyte and fibroblast proliferation, endothelial cell migration and re-epithelization. All these processes are incorporated in wound healing. IGF strengthens the wound [27]. On the other hand, IGF-1 expression is decreased in diabetic wounds which leads to defective cell granulation. This may be attributed to the acidic nature of the wound environment which affects its affinities [28].

3.6. RT-PCR results

The gene expression of IGF was evaluated in the diabetic and stem cells groups on day 14 using RT-PCR as shown inTable 2, Fig. 5. The diabetic group revealed marked decrease in gene expression of IGF when compared to stem cells group. Moreover, the stem cells group showed marked increase in IGF levels as compared to diabetic group.IGF is formed of two peptides IGF-1 and IGF-2. IGF-1

Table 2. The mean values of EGF and IGF mRNAexpression levels ±SD, on day14 post wound induction

Groups		Diabetic group	Stem cells group
Day 14	EGF	0.23 ± 0.08	3.06 ± 0.07
	IGF	0.12 ± 0.09	2.81 ± 0.18

In addition, the gene expression of EGF was assessed in the diabetic and stem cells groups on day 14 using RT-PCR as shown inTable 2, Fig. 5. The diabetic group revealed noticeable decrease in gene expression of EGF as compared to stem cells group. On the other hand, the stem cells group showed significant increase in EGF levels when compared to diabetic group.EGF proteins are produced by fibroblasts, platelets and macrophages and confine throughout the epidermis, mainly in the basal layer.EGF serves as the stimulus for epithelial proliferation[29].

ADSCs secrete high levels of growth factors.They can enhance tissue regeneration by differentiating into skin cells and by secretion of paracrine factors, which can promote the healing process via regulating the inflammatory response[23].

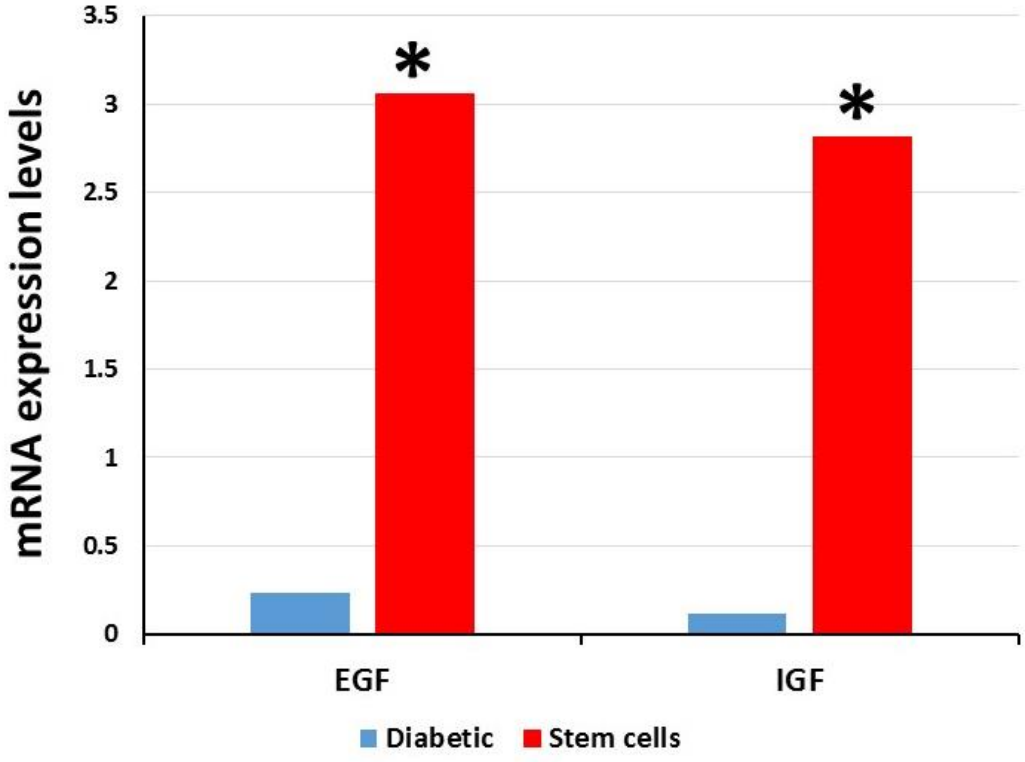

Fig. 5. Displaying RT-PCR analysis of EGF and IGF in the diabetic and stem cells groups at day 14 after wound induction, *: significant to diabetic group (P value < 0.05).

M.M. Allam, A.S. Farid, B.A. Saffaf, D. Sabry, Adipose mesenchymal stem cells combined with platelet-rich plasma accelerate diabetic wound healing by modulating the Notch pathway, Stem Cell Res. Ther. 12 (2021) 1–24.

[10] Z. Zhang, L. Lv, Effect of local insulin injection on wound vascularization in patients with diabetic foot ulcer, Exp. Ther. Med. 11 (2016) 397–402. https://doi.org/10.3892/etm.2015.2917.

[11] S.S. Kamar, D.H. Abdel-Kader, L.A. Rashed, Beneficial Effect of Curcumin Nanoparticles-Hydrogel on Excisional Skin Wound Healing in Type-I Diabetic Rat: Histological and Immunohistochemical Studies, Ann. Anat. 222 (2019) 94–102. https://doi.org/10.1016/j.aanat.2018.11.005.

[12] M. Rezvanian, S.F. Ng, T. Alavi, W. Ahmad, In-vivo evaluation of Alginate-Pectin hydrogel film loaded with Simvastatin for diabetic wound healing in Streptozotocin-induced diabetic rats, Int. J. Biol. Macromol. 171 (2021) 308–319. https://doi.org/10.1016/j.ijbiomac.2020.12.221.

[13] W.S. Tan, P. Arulselvan, S. Ng, C.N.M. Taib, M.N. Sarian, S. Fakurazi, Improvement of diabetic wound healing by topical application of Vicenin-2 hydrocolloid film on Sprague Dawley rats, BMC Complement. Altern. Med. 19 (2019) 1–16.

[14] Y. Shao, M. Dang, Y. Lin, F. Xue, Evaluation of wound healing activity of plumbagin in diabetic rats, Life Sci. 231 (2019) 116422. https://doi.org/10.1016/j.lfs.2019.04.048.

[15] Y. Zheng, S. Zheng, X. Fan, L. Li, Y. Xiao, P. Luo, Y. Liu, L. Wang, Z. Cui, F. He, Y. Liu, S. Xiao, Z. Xia, Amniotic Epithelial Cells Accelerate Diabetic Wound Healing by Modulating Inflammation and Promoting Neovascularization, Stem Cells Int. 2018 (2018) 10.

[16] H. Shamsi, M. Velayati, H. Rahimzadeh, N. Mozafari, Beneficial Effects of Nanocurcumin Loaded Chitosan Biofilm on Healing of Full Thickness Excisional Wounds in Diabetic Rats, Iran. J. Vet. Surg. 15 (2020) 32–41. https://doi.org/10.30500/ivsa.2020.208499.1203.

[17] K. J.A., Histological and Histochemical methods: Theory and Practice, in: 5th

4. Conclusion

The present study provided experimental evidence that the ADSCsenhance diabetic wound healing through increasing gene expression of insulin like growth factor and epidermal growth factor. Also they enhance epithelialization and collagen formation. Further studies needed to examine the other mechanisms by which ADSCs accelerate wound healing.

5. References

[1] L. Deng, C. Du, P. Song, T. Chen, S. Rui, D.G. Armstrong, W. Deng, Molecular Mechanisms of Dietary Bioactive Compounds in Redox Balance and Metabolic Disorders, Oxid. Med. Cell. Longev. 2021 (2021) 1–11.

[2] Y. An, S. Lin, X. Tan, S. Zhu, F. Nie, Y. Zhen, L. Gu, C. Zhang, B. Wang, W. Wei, D. Li, J. Wu, Exosomes from adipose-derived stem cells and application to skin wound healing, (2021) 1–12. https://doi.org/10.1111/cpr.12993.

[3] Q. Bai, K. Han, K. Dong, Q. Long, Potential Applications of Nanomaterials and Technology for Diabetic Wound Healing, (2020) 9717–9743.

[4] D.E. Rodríguez-Fuentes, L.E. Fernández-Garza, J.A. Samia-Meza, S.A. Barrera-Barrera, A.I. Caplan, H.A. Barrera-Saldaña, Mesenchymal Stem Cells Current Clinical Applications: A Systematic Review, Arch. Med. Res. 52 (2021) 93–101. https://doi.org/10.1016/j.arcmed.2020.08.006.

[5] A. Arceri, A. Mazzotti, E. Artioli, S.O. Zielli, F. Barile, M. Manzetti, G. Viroli, A. Ruffilli, C. Faldini, Adipose-derived stem cells applied to ankle pathologies: a systematic review, Musculoskelet. Surg. (2023). https://doi.org/10.1007/s12306-023-00798-7.

[6] M.S. Hu, M.R. Borrelli, H.P. Lorenz, M.T. Longaker, D.C. Wan, Review Article Mesenchymal Stromal Cells and Cutaneous Wound Healing : A Comprehensive Review of the Background , Role , and Therapeutic Potential, 2018 (2018).

[7] W.M. Jackson, L.J. Nesti, R.S. Tuan, Mesenchymal stem cell therapy for attenuation of scar formation during wound healing, (2012).

[8] J.L. Burgess, W.A. Wyant, B.A. Abujamra, R.S. Kirsner, I. Jozic, Diabetic Wound-Healing Science, Medicina (B. Aires). 57 (2021) 1072.

[9] N. Ebrahim, A.A. Dessouky, O. Mostafa, A. Hassouna, M.M. Yousef, Y. Seleem, E. Abd, E. Aziz, M. El Gebaly,

Ramirez, A.G. Nusbaum, A. Sawaya, S.B. Patel, L. Khalid, R.R. Isseroff, M. Tomic-Canic, Epithelialization in Wound Healing: A Comprehensive Review, Adv. Wound Care. 3 (2014) 445–464. https://doi.org/10.1089/wound.2013.0473.

[27] Z.O.E. Garoufalia, A. Papadopetraki, E. Karatza, D. Vardakostas, A. Philippou, G. Kouraklis, D. Mantas, Insulin-like growth factor-I and wound healing , a potential answer to non-healing wounds : A systematic review of the literature and future perspectives, (2021) 1–5. https://doi.org/10.3892/br.2021.1442.

[28] S. Patel, S. Srivastava, M.R. Singh, D. Singh, Biomedicine & Pharmacotherapy Mechanistic insight into diabetic wounds : Pathogenesis , molecular targets and treatment strategies to pace wound healing, Biomed. Pharmacother. 112 (2019) 108615. https://doi.org/10.1016/j.biopha.2019.108615.

[29] S. Yamakawa, K. Hayashida, Advances in surgical applications of growth factors for wound healing, 1 (2019) 1–13.

ed., Scion Publishing Ltd, 2015: pp. 144–146.

[18] N.A. Ghazali, A. Elmy, L.C. Yuen, N.Z. Sani, S. Das, F. Suhaimi, R. Yusof, N.H. Yusoff, Z.C. Thent, Piper betel leaves induces wound healing activity via proliferation of fi broblasts and reducing 11 b hydroxysteriod dehydrogenase-1 expression in diabetic rat, J. Ayurveda Integr. Med. 7 (2016) 198–208. https://doi.org/10.1016/j.jaim.2016.08.008.

[19] A. Al-romaima, X. Guan, X. Qin, Y. Liao, G. Qin, S. Tang, J. Feng, Topical Application of Chinese Formula Yeliangen Promotes Wound Healing in Streptozotocin-Induced Diabetic Rats, J. Diabetes Res. 2022 (2022) 1014.

[20] M.C. Deeds, J.M. Anderson, A.S. Armstrong, D.A. Gastineau, H.J. Hiddinga, A. Jahangir, N.L. Eberhardt, Y.C. Kudva, Single dose streptozotocin-induced diabetes: Considerations for study design in islet transplantation models, Lab. Anim. 45 (2011) 131–140. https://doi.org/10.1258/la.2010.010090.

[21] M. Gadelkarim, A.I. Abushouk, E. Ghanem, A.M. Hamaad, A.M. Saad, M.M. Abdel-Daim, Adipose-derived stem cells: Effectiveness and advances in delivery in diabetic wound healing, Biomed. Pharmacother. 107 (2018) 625–633. https://doi.org/10.1016/j.biopha.2018.08.013.

[22] N.S. Younis, M.E. Mohamed, N.A. El Semary, Green Synthesis of Silver Nanoparticles by the Cyanobacteria Synechocystis sp.: Characterization, Antimicrobial and Diabetic Wound-Healing Actions, Mar. Drugs. 20 (2022) 56. https://doi.org/10.3390/md20010056.

[23] W.U. Hassan, U. Greiser, W. Wang, Role of adipose-derived stem cells in wound healing, Wound Repair Regen. 22 (2014) 313–325. https://doi.org/10.1111/wrr.12173.

[24] B.E. Aboulhoda, S. Abd el Fattah, Bone marrow-derived versus adipose-derived stem cells in wound healing: value and route of administration, Cell Tissue Res. 374 (2018) 285–302. https://doi.org/10.1007/s00441-018-2879-x.

[25] S. Azizi, R. Kheirandish, M. Salarpoor, Topical effect of allogenous serum rich in growth factors (SRGF) on diabetic skin wound in rat, Transfus. Apher. Sci. 58 (2019) 498–504. https://doi.org/10.1016/j.transci.2019.05.014.

[26] I. Pastar, O. Stojadinovic, N.C. Yin, H.