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RESEARCH

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Overexpression of miR-192 in fibroblasts accelerates wound healing in diabetic rats: research article

Forouzan Karam[1], Mahtab Sayadi[1], Saeedeh Dadi[1] and Gholamreza Anani Sarab[1*]

Abstract

Background Diabetic foot ulcer (DFU) is a severe diabetic complication. Transplantation of skin substitutes, stem cells, and platelet-rich plasma (PRP) treatments are promising tools to promote ulcer healing in diabetes. An important aspect of the remodeling phase of wound healing is collagen deposition. miR-192 increases the expression of *COL1A2* by specifically targeting Smad-interacting protein 1 (SIP1). This study was designed to investigate the impact of combined treatment with platelet-rich plasma and fibroblast cells expressing miR-192 on the healing process of wounds using an experimental diabetic animal model.

Methods After transfection of HDF cells and induction of increased miR-192 expression, relative changes in *COL1A2* gene expression were determined by the RT-PCR method. Rats were randomly divided into 6 groups: non-diabetic control group, diabetic control, backbone, PRP, miR-192, and PRP + miR-192 groups. Diabetes was induced in male Wistar rats of all treated groups except non-diabetic control through a 21-day high-fat diet and an intraperitoneal injection of 40 mg/kg streptozotocin. A 10-mm skin biopsy punch was used to create two full-thickness wounds on the dorsal part of the upper body in all six groups of animals. Hematoxylin–eosin and Mason's trichrome staining were used to evaluate the wounds and analyze histological changes.

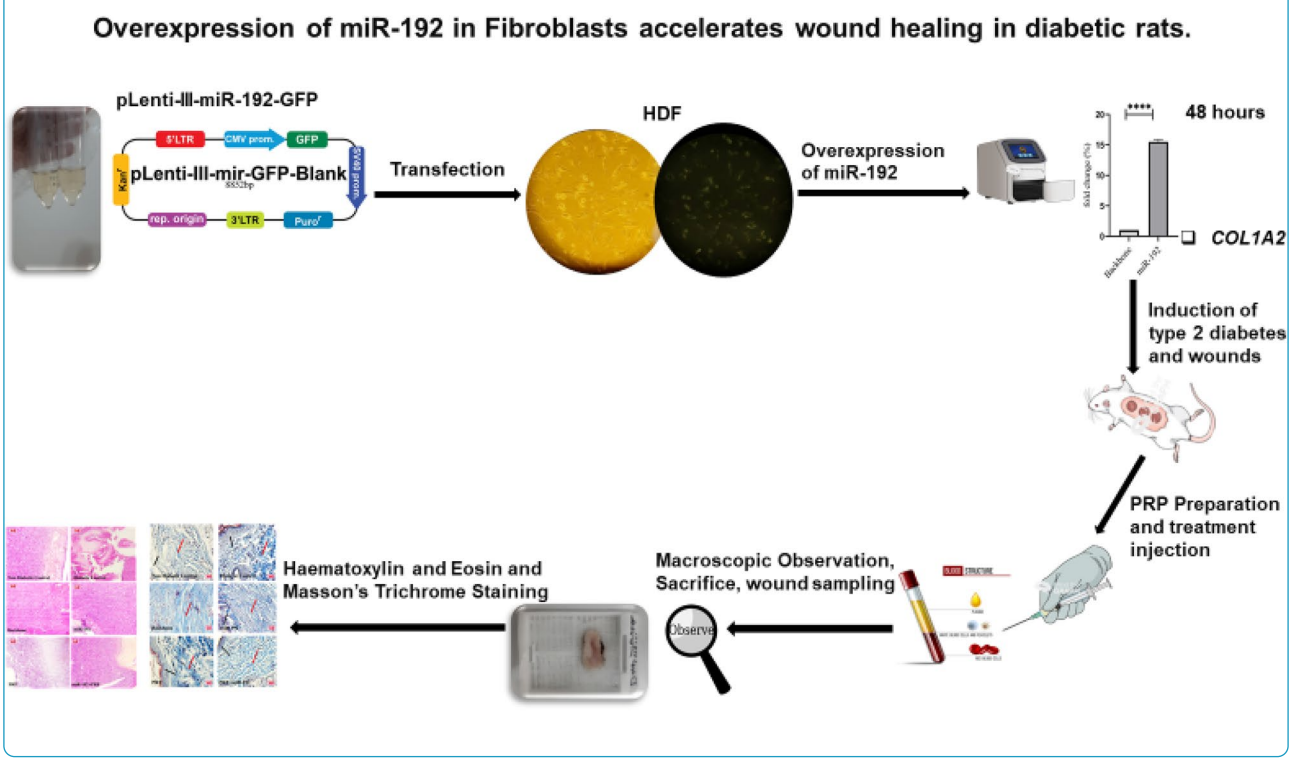
Results The overexpression of miR-192 in HDF cells resulted in a significant increase in *COL1A2* gene expression, which was 15.77-fold higher than the control group. PRP and pLenti-III-miR-192-GFP-expressing cells significantly increased wound closure rates, granulation tissue area, and collagen fiber density in rats, according to a histological examination.

Conclusion The combined use of PRP and HDFs expressing pLenti-III-miR-192-GFP speeds up the healing of wounds by increasing collagen expression, demonstrating the efficacy of this approach in improving wound healing results.

Keywords Diabetes, Diabetic foot ulcer, Fibroblast, miR-192, Platelet-rich plasma, Wound healing

*Correspondence:
Gholamreza Anani Sarab
ghansa@yahoo.com

Graphical Abstract



Background

Diabetes mellitus (DM) is a widespread health condition impacting approximately 9.3% of the global population [1]. Diabetic foot ulcer (DFU) is a prevalent chronic complication of diabetes with significant medical, economic, and social impacts. Research suggests that 15–25% of diabetic individuals are at risk of developing foot ulcers during their lifetime [2].

The stages of wound healing include homeostasis, which occurs immediately after wound formation, inflammation, which occurs from 10 to 15 min to 3 days after injury, proliferation, which occurs from 4 to 21 days after injury, and regeneration, which occurs from 21 days to a year [3, 4].

Fibroblasts secrete growth factors, collagen, and other elements of the extracellular matrix (ECM), which are vital for wound healing. They release platelet-derived growth factor (PDGF), fibroblast growth factors (FGF), and transforming growth factor (TGF) to promote cell division, activity, or differentiation [5–8]. Platelet-rich plasma (PRP) is considered a natural growth factor that is safe to use in enhancing the healing rate of wounds. It is particularly effective in treating chronic wounds associated with diabetes that require prompt repair to prevent infections. One clinical application is the use of PRP gel for chronic ulcers [9–11]. PRP is a product of blood plasma with a high platelet concentration that contains numerous growth factors and cytokines, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor [12]. More importantly, PRP can enhance the proliferation and migration of dermal fibroblasts, indicating that it may synergistically heal chronic wounds [13].

MicroRNAs (miRNAs) are small RNA molecules that are approximately 19 to 22 nucleotides in length. They regulate gene expression at the post-transcriptional level by binding to the untranslated 3' regions (UTR) of target messenger RNA (mRNA) molecules [14]. Multiple miRNAs have been identified in skin tissue, and they are believed to participate in various biological processes, including the regulation of wound healing [15–17]. The expression of miR-29a has been found to have a direct impact on collagen expression, whereas miR-192, miR-29b, and miR-29c are significantly upregulated during this process [18–21]. miR-192 promotes the expression

Table 1 Primers used in this study

Oligo name	Sequence 5–3	BC	EC	GC%	MW (Da)	*TM (℃)	OD 260 nm	Product length	Oligo ID/metabion international AG
Forward primer COL1A2	TCT CTA CTG GCG AAA CCT GTA	21	197,0	47.62	6.381	59	3,5	98	220823B003H05 1/2
Reverse primer COL1A2	TCC TAG CCA GAC GTG TTT CTT	21	189,0	47.62	6.363	59	4,3	98	220823B003A06 2/2

of *COL1A1* by targeting smad-interacting protein 1 (SIP1) [22]. While current treatments for wound care have made significant progress, the replacement of lost skin remains a major challenge in the field of regenerative medicine. Despite several available treatment methods for wound healing, a treatment approach that accelerates the healing process would be highly valuable [23].

To our knowledge, relevant research on the combination of fibroblasts with overexpression of miR-192 and PRP for the treatment of wound healing is very limited. This research investigated the impact of co-treatment with fibroblasts expressing miR-192 and platelet-rich plasma in the healing of wounds in a diabetic rat model.

Materials and methods

Cell lines and culture

Primary human dermal fibroblasts (obtained from the laboratory of Dr. Mohsen Khorashadizadeh) were cultured in DMEM (BIO-IDEA, Tehran, Iran) with 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin and streptomycin and incubated at 37 °C in a 95% humidified incubator with 5% CO2.

Plasmids construct and extraction

The pLenti-III-pre-miR192-GFP (green fluorescent protein) expression vector construct and pLenti-III-Backbone-GFP (mock) were purchased from ABM Inc (Applied Biological Materials, Richmond, BC, Canada). E. coli Stbl4 strain harboring the vectors was cultured in Luria–Bertani (LB) broth medium with 50 µg/mL kanamycin. The plasmid was extracted with the Karmania Pars Gene plasmid extraction kit (Karmania Pars Gene, Kerman, Iran).

Transient transfection

For each well, 2×10^5 cells were seeded in a 6-well cell culture plate. Separately, 3 µg of plasmids and 4 µl of PEI in 100 µl of DMEM medium were dissolved in two 1.5-ml Microtubes, the mixture was added dropwise to the cells, 6 h later medium was replaced with fresh medium.

Confirmation of GFP expression by flow cytometry and fluorescence microscope

Forty-eight hours after the transfection, the expression of the labeled GFP protein in the cells was verified by trypsinizing the cells and analyzing them using a flow cytometry system. The cells were first diluted in PBS buffer at 3×10^4 cells per tube and homogenized using a vortex. The samples were then analyzed using the FL1 channel of the flow cytometry (CYFLOW CUBE 8, Sysmex, Germany). Additionally, to evaluate the expression of GFP, fluorescence microscopy (Olympus BX41TF, Japan) analysis was performed on transfected human dermal fibroblasts.

RNA extraction

Forty-eight hours post-transfection, total RNA was extracted from HDF cells using RNX-plus reagent (Sinaclon, Tehran, Iran) according to the manufacturer protocol. The extracted RNAs were then quantified using Nanodrop spectrophotometry (BioTek Epoch microplate spectrophotometer, USA) by measuring the

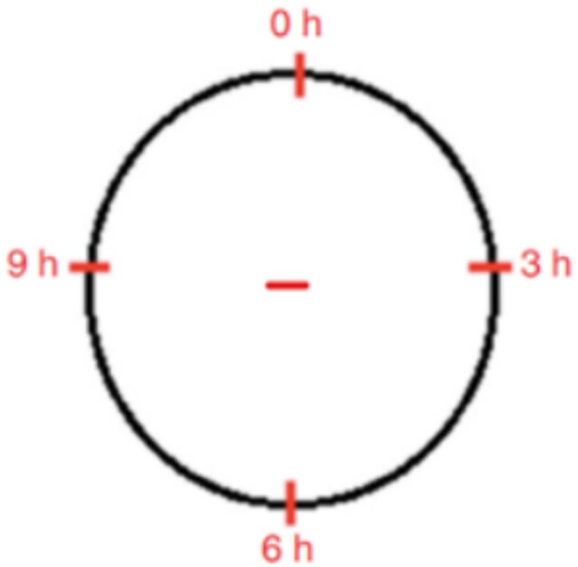


Fig. 1 Schematic figure of cell and PRP injection site. Treatments were injected at the center of circular wounds, positions 12, 3, 6, and 9

absorbance at 260/280 and 260/230 nm wavelengths, Samples were kept at −80 °C until use.

cDNA synthesis and stem-loop qPCR of miR-192

Stem-loop primers and the miR-192-5P specific primer used was 5′- CTCTGACTTATGAATTGAC-3′ (forward) and reverse primers were designed to synthesize the cDNA of miR-192 according to the BON Stem High Sensitivity microRNA 1st Strand cDNA Synthesis kit protocol (Stem Cell Technology Research Center, Tehran, Iran) for qPCR, RT-qPCR was performed using StepOne™ Real-Time PCR System (StepOne™ Real-Time PCR System, USA) under the following conditions: 95 °C for 2 min, and 60 °C for 30 s for up to 40 cycles. U6 primer used was 5- AAGGATGAC ACGCAAAT-3 (forward) and was utilized as an internal control to normalize the RNA input. The relative expression of miR-192 was evaluated using the ΔΔCT method, the experiment was performed in triplicate ($n=3$).

RT-qPCR for collagen 1A2 gene expression

The extracted RNA was reverse transcribed into cDNA using the Easy cDNA Synthesis Parstous Kit from Parstous, Mashhad, Iran. The RT-qPCR reactions were carried out using RealQ plus 2×Master Mix Green (AMPLIQON, Denmark) The experiment was conducted on a Real-time PCR System (StepOne™ Real-Time PCR System, USA) under the following conditions: 95 °C for 2 min, and 61 °C for 30 s for up to 40 cycles.

The primers used to target the *COL1A2* gene were 5′GAGGGCAACAGCAGGTTCACTTA-3′ (forward) and 5′-TCAGCACCACCGATGTCCAA-3′ (reverse) (Table 1), *GAPDH* was used as an internal control gene. The ΔΔCT method was used for calculation ($n=3$).

PRP preparation

PRP was collected from five healthy male and female donors, aged 25–30 years with an average initial platelet count of 300,000. Blood with sodium citrate anticoagulant (50 ml) was prepared and centrifuged at 1200 rpm for 10 min. The supernatant was separated and re-centrifuged at 3000 rpm for 10 min. The platelet precipitation was resuspended in the plasma. After concentration increased to an average of 800,000 to 900,000 platelets, which was counted using the gold standard platelet counting method with a hemocytometer.

Experimental animals and study design

8- to 10-week-old male Wistar rats were obtained from the Pasteur Institute of Iran (Tehran, Iran) for this study. Animals were kept in standard cages with four rats per cage. The rats were kept at a controlled room temperature of 22 ± 2 °C and 60 ± 5% humidity. Animals were exposed to a 12-h light–dark cycle and standard food and water. Rats weighing about 180–200 g were randomly divided into six groups with eight rats in each group:

Group 1: wound model in non-diabetic rats; group 2: wound model in diabetic rats, group 3: wound model in diabetic rats treated with 100 µl of PRP; group 4: wound model in diabetic rats treated with 6×10^4 HDF expressing pLenti-III-Backbone-GFP suspended in 100 µl of DMEM medium; group 5: wound model in diabetic rats treated with 6×10^4 HDF expressing pLenti-III-miR-192-GFP suspended in 100 µl of DMEM medium; group 6: wound model in diabetic rats + 100 µl of PRP + 6×10^4 HDF expressing pLenti-III-miR-192-GFP.

Induction of type 2 diabetes in rats

The experiments were performed following institutional guidelines for animal care and approved by the

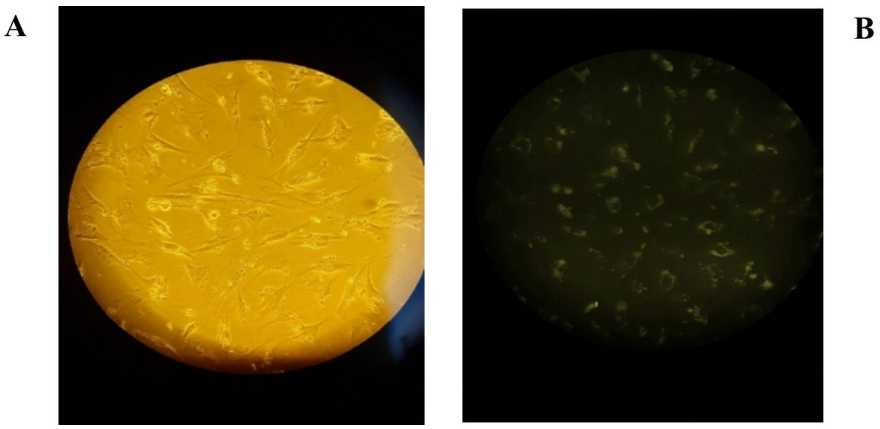


Fig. 2 GFP expression in human skin fibroblast cells. Magnification × 40. **A** HDF cells expressing GFP with visible light. **B** HDF cells expressing GFP with fluorescent light

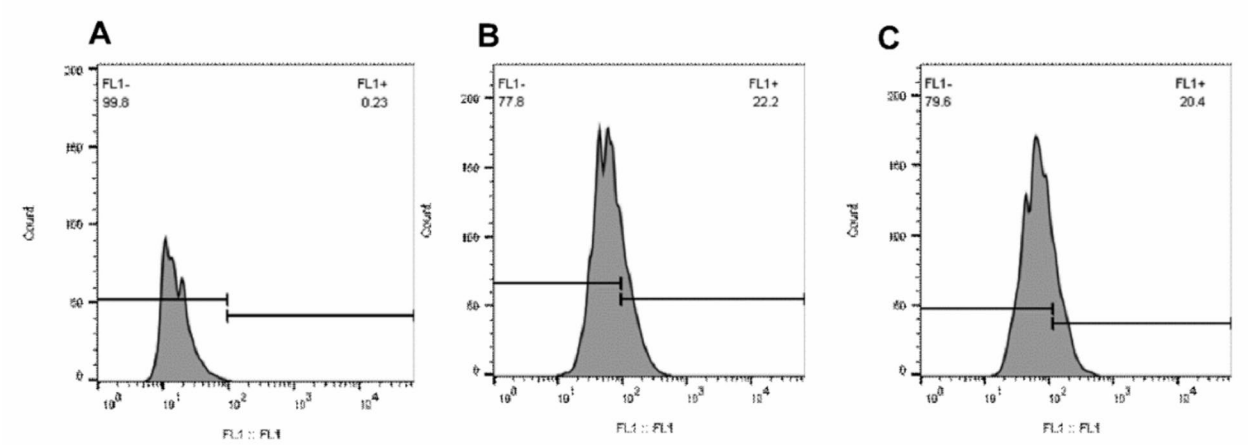


Fig. 3 Percentage of HDF cells transfected with pLenti-III-miR-192-GFP and pLenti-III-Backbone-GFP versus the negative control group by flow cytometry. **A** negative control group with fresh HDF (GFP: 0.2% positive). **B** HDF group expressing pLenti-III-miR-192-GFP (GFP: 22% positive). **C** HDF group expressing pLenti-III-Backbone-GFP (GFP: 20% positive)

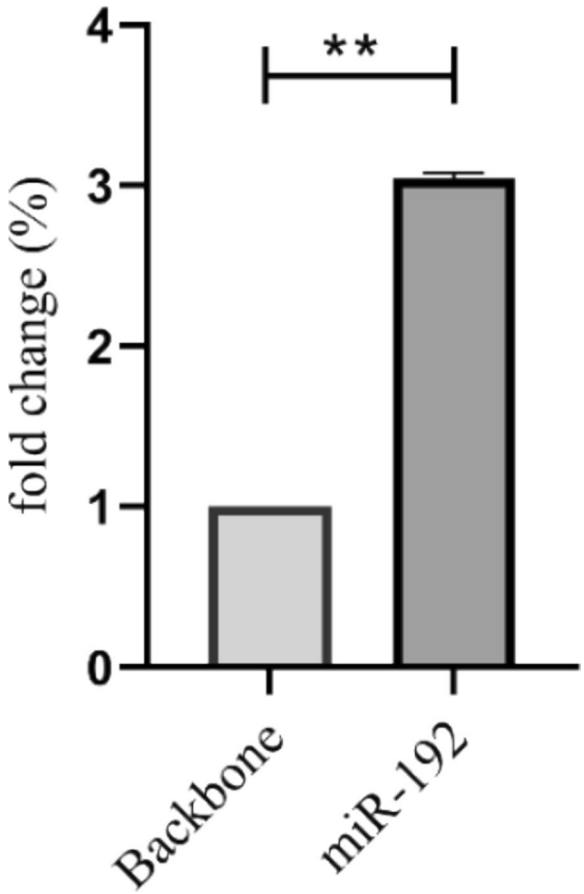


Fig. 4 Increased expression of miR-192 in human skin fibroblast cell line. 48 h after transfection with miR-192 expressing plasmid and backbone expressing plasmid, the level of miR-192 in both groups of cells was measured by RT-qPCR. ($P = 0.0078$) **. ($n = 3$)

local ethics committee (IR.BUMS.REC.1400.413). Type 2 diabetes was induced in rats using the fat-fed streptozotocin (ZellBio GmbH, Berlin, Germany) (STZ) model mouse protocol. The rats were placed on a high-fat diet for 3 weeks, providing 60% of their caloric value as fat [24], on day 22, all rats were fasted for 8 h before STZ injection, and fasting blood glucose was measured, 40 mg of STZ was weighed, transferred to a 1.5-ml microcentrifuge tube and covered with aluminum foil, Immediately before injection, citrate buffer was prepared, STZ was dissolved in 50 mM sodium citrate buffer at pH 4.5 to a final concentration of 40 mg/ml, administered within 5 min after dissolution, using a 1-ml syringe and 23-G needle, STZ was injected intraperitoneally (i.p.) into the test group at a dose of 40 mg/kg of body weight (1.0 ml/kg). An equal volume of citrate buffer with pH 4.5 was injected i.p. into the control animals. For the diabetic groups, the rats received high-fat food, whereas the control group had a typical diet. Ten days following STZ delivery, blood glucose levels were assessed using the Infopia EasyGluco (Autocoding blood glucose meter EasyGlucoTM, South Korea) in a tail vein blood sample. Blood glucose levels exceeding 15 mmol/L (270 mg/dL) indicated that STZ-treated rats had successfully induced type 2 diabetes [24].

Experimentally induced wounds and treatments

Rats were anesthetized intraperitoneally using ketamine (Alfasan, Woerden, Holland) and xylazine (KELA.N.V, Belgie), and then the dorsal aspect of the upper part of the body was shaved and disinfected with 70% alcohol. A 10-mm skin biopsy punch was induced to create full-thickness wounds under aseptic

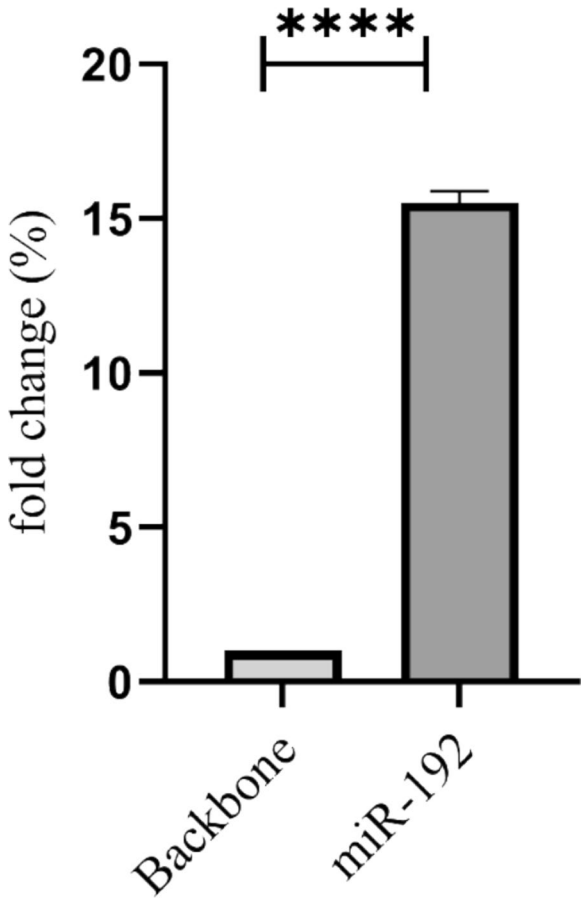


Fig. 5 Analysis of *COL1A2* gene expression in cells transfected with miR-192 expressing plasmids against the control group transfected with backbone expressing plasmids. **** ($P < 0.0001$). ($n = 3$)

conditions, treatments were performed in each group according to the previously mentioned protocol, in the form of subcutaneous injections in 5 points according to Fig. 1. Animals were killed on the 3rd, 7th, 14th and 21st days after wounding.

Macroscopic observation

Wounds were measured using a digital caliper and photographed on the day of wounding and subsequently on alternate days until healing was complete. Changes in wound areas were calculated at each time point to monitor the rate of wound contraction, the percentage reduction in wound size was calculated using the following equation [25]:

$$\%\ \text{wound healing} = \frac{\text{Wound area day 0} - \text{ wound area days (3/7/14/21)} \times 100}{\text{Wound area day 0}}$$

Hematoxylin and eosin and Masson's trichrome staining

The wound area was removed using a sharp sterile scalpel. Then the cut tissue was fixed in a 10% formalin solution to preserve its structure and prevent destruction. The tissue was dehydrated through a series of alcohol solutions with increasing concentration.

The tissue was cleared using xylene, tissue infiltration and embedding were performed using paraffin wax or other embedding materials to support the tissue during sectioning, then the histological sections were cut using a microtome and placed on glass slides, slides were stained with hematoxylin, eosin, and Masson's trichrome to observe collagen deposition and other histological features. An optical microscope (Microscope camera ODC series, England) was used to observe and photograph the slides, and all histological examinations were performed by two pathologists blindly.

Statistical analysis

In this study, Fiji/ImageJ 2.9.0 software was used for quantitative analysis of the density of collagen fibers, the number of fibroblast cells, and the amount of granulation during the different stages of wound healing. The data obtained from the Fiji/ImageJ software were analyzed and the results were presented using graphs and charts drawn with GraphPad Prism 9 software. The statistical significance of the data was determined using the two-way ANOVA and t-$tudent's test, and a significance level of p value less than 0.05 was considered in all experiments.

Results

Expression of GFP-tag and observation of green color as confirmation for transfection of expression plasmids in human dermal fibroblast cells

The transfection of HDF cells with pLenti-III plasmids was successful with a 20–25% transaction rate as estimated after 48 h of transfection. The confirmation of the transfection was done using a fluorescence microscope, and the results are presented in Fig. 2.

The expression level of miR-192 was analyzed by flow cytometry using the FL1 channel compared to fresh and untransfected cells, the results of this analysis are presented in Fig. 3 of the study.

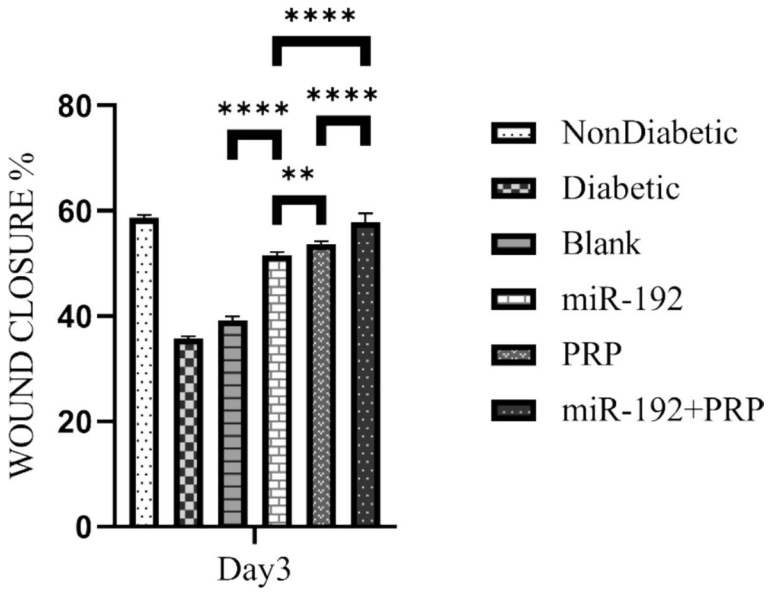


Fig. 6 Comparing the rate of wound closure on the third day after surgery, between the intervention groups with each other. NS (not significant) and * ($P<0.05$) and ** ($P<0.01$) and *** ($P<0.001$) and **** ($P<0.0001$)

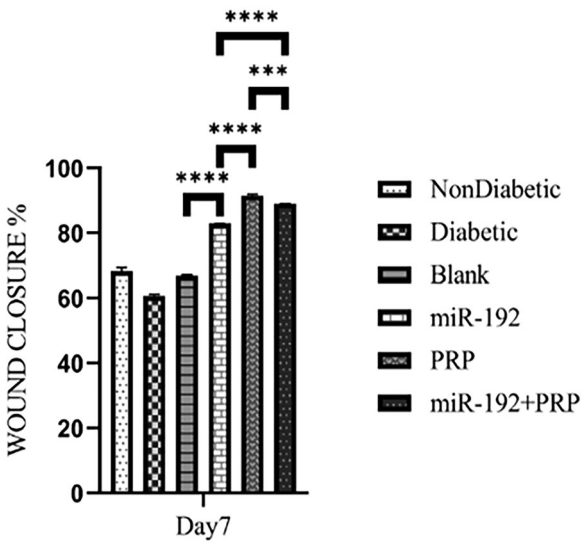


Fig. 7 Comparing the rate of wound closure on the seventh day after surgery, between the intervention groups with each other. NS (not significant) and * ($P<0.05$) and ** ($P<0.01$) and *** ($P<0.001$) and **** ($P<0.0001$)

Expression of miR-192 in transfected fibroblast cells increased COL1A2 gene expression

The results of this analysis showed a significant increase (threefold) in miR-192 expression in the fibroblast cells transfected with the miR-192 expressing plasmid compared to the cells transfected with the backbone expressing plasmid after 48 h, as shown in Fig. 4, this confirmed the successful transfection of the cells and the overexpression of miR-192 in the transfected cells.

The study used RT-qPCR to test if miR-192 increased in fibroblast cells would change *COL1A2* expression, Results showed a 15.77-fold increase in *COL1A2* expression in transfected cells compared to the control group after 48 h (Fig. 5), suggests miR-192 positively regulates *COL1A2* expression and a potential mechanism for its effects on wound healing.

Simultaneous treatment with HDF cells expressing miR-192 and PRP caused more wound closure than other groups

On the 3rd day, a significant difference was seen among the group receiving HDFs expressing pLenti-III-Backbone-GFP and the group treated with HDFs expressing pLenti-III-miR-192-GFP ($P<0$ /0001).There was a significant difference between the group that received PRP and HDFs expressing pLenti-III-miR-192-GFP and other treatment groups $P<0.0001$ and more wound closure have occurred (Fig. 6).

On the seventh day after wound formation, the wounds were examined macroscopically, and measurements were taken and recorded with a digital caliper. On the seventh day, the wound healing process accelerated in all intervention groups compared to the third day, and the difference between the groups became more obvious. According to the order of the groups in Fig. 7, the average percentage of wound closure in various intervention

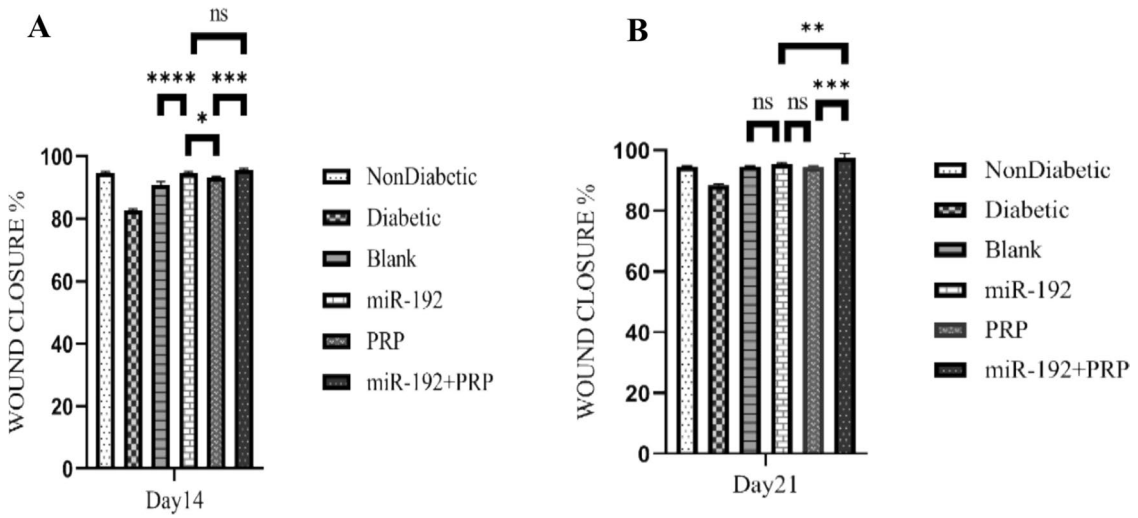


Fig. 8 Comparing the rate of wound closure on days 14 and 21 after wound formation between the intervention groups. **A** Comparing the rate of wound closure on day 14. **B** Comparing the rate of wound closure on day 21. NS (not significant) and * ($P<0.05$) and ** ($P<0.01$) and *** ($P<0.001$) and **** ($P<0.0001$)

groups versus the control groups is as follows: 68/33, 60/67, 66/83, 83/00, 91/53, and 89/03 percent.

A significant difference of $P=0.0001$ was seen in the healthy control group and the miR-192-expressing, PRP-group, and combined treatment groups, and these groups healed wounds quicker than the healthy control group. On the seventh day, the diabetic control group that did not receive treatment experienced a delay in the healing of the wound compared to the other groups. The difference between the rates of wound closure in the treated groups compared to the diabetic control group was almost completely different in the treated groups. Most of the wound area is closed, especially in the fifth and sixth groups, where it ranges from 80 to 90%, with diabetic wounds being close to 60%. In comparison to the group that only received fibroblast cells expressing pLenti-III-miR-192-GFP, more wounds closed in the combination therapy group ($P=0.0001$) (Fig. 7).

On the 14th day, there was a significant difference between the HDF groups expressing pLenti-III-Backbone-GFP and pLenti-III-miR-192-GFP plasmids ($P<0.0001$), and more wound closure was observed in the HDF group expressing pLenti-III-miR-192-GFP. Between the PRP-treated group and the HDF-expressed group, there was a smaller difference ($P<0.05$). The group that received the combination therapy with the groups treated with PRP and fibroblasts experiencing pLenti-III-miR-192-GFP alone is significant, with $P<0.0001$ and NS (not significant), respectively.

On the 21st day after wound formation, healing occurred in almost all intervention groups, but the diabetic group without treatment has the lowest average percentage of wound closure (88.48%) compared to other groups, the group that simultaneously received PRP and fibroblast cells expressing pLenti-III-miR-192-GFP showed significant differences with each group that received the mentioned treatments alone, the group that was treated with combined treatment, the rate of wound closure and scars left from healing was lower than in other groups (Fig. 8). The macroscopic views of the wound on different days are shown in Fig. 9.

The number of fibroblast cells in the group that simultaneously received HDF cells expressing miR-192 and PRP was higher than in the other groups

On the 14th and 21st days following the development of a wound, the average number of fibroblast cells was counted in the dermis of the wound area in various intervention groups. Figure 10 shows that the group that received the combined treatment had more fibroblast cells than the other groups. The fact that there was a noticeable difference between this group and the groups which received each treatment separately could mean that the injected fibroblast cells in the wound area were functional and alive.

The density of collagen fibers in the group treated with PRP and HDF cells expressing miR-192 increased compared to other groups

The density of collagen fibers in the group treated with PRP and HDF cells expressing miR-192 increased compared to other groups the group that got the combination

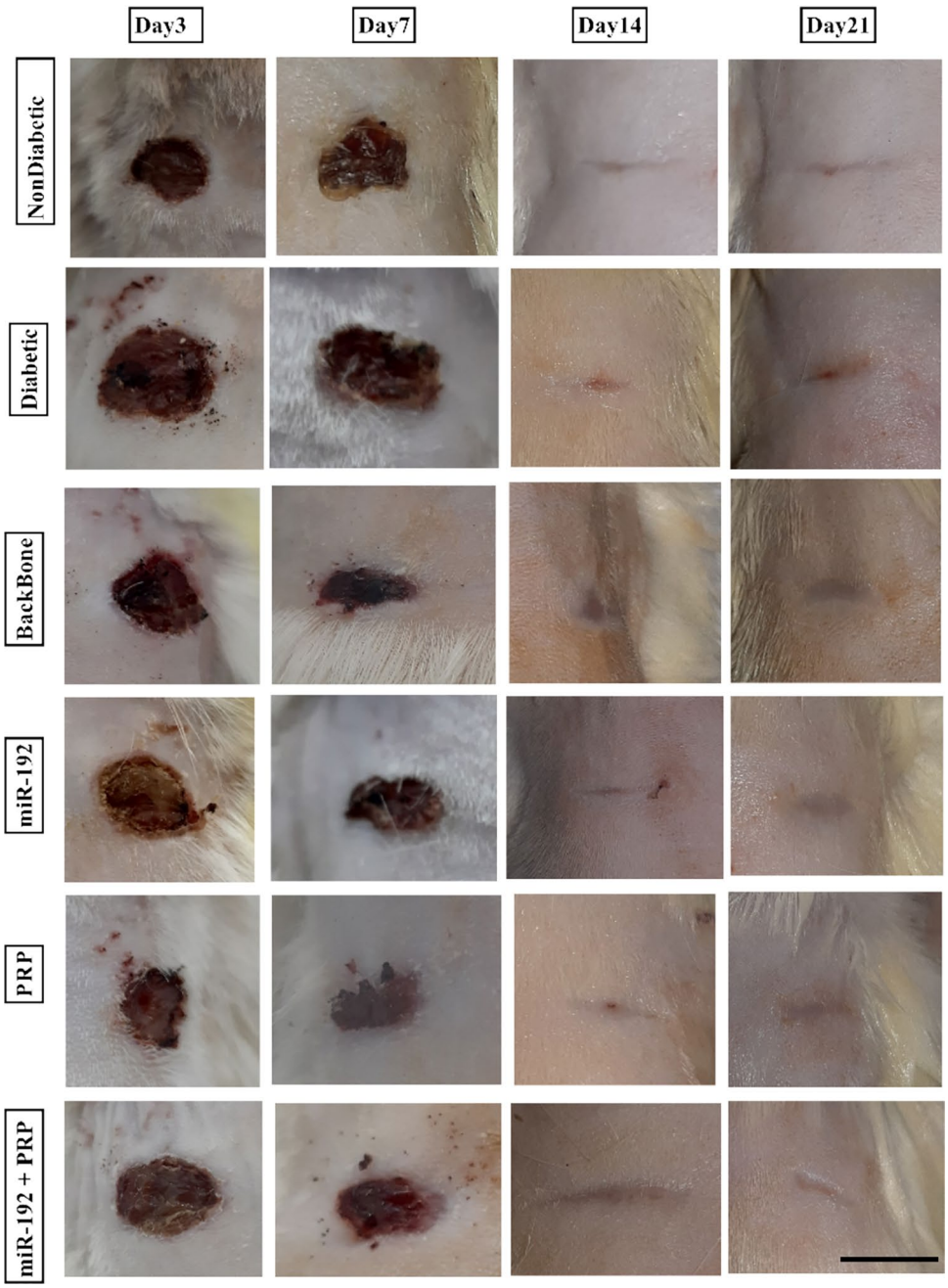


Fig. 9 Macroscopic views of wounds on different days. The wound pictures of the rats' dorsal skin of the Non-Diabetic control group, Diabetic control and intervention groups were taken on post-injury days 3, 7, 14, and 21. Scale bar = 1 cm

therapy and HDF expressing pLenti-III-miR-192-GFP had a higher collagen fiber density on day 14 than the other groups, and there was a significant difference between these two groups and the PRP-treated group. A notable difference was observed between the groups that got HDF containing pLenti-III-miR-192-GFP plasmid and the backbone group on the 21st day following injury, additionally compared to each of the treatment groups separately, the combined treatment group's collagen fiber density increased, however, this rise was not statistically significant (Fig. 11).

Figure 12 shows Masson-trichrome staining of the wound area in different groups on the 14th day after wound induction.

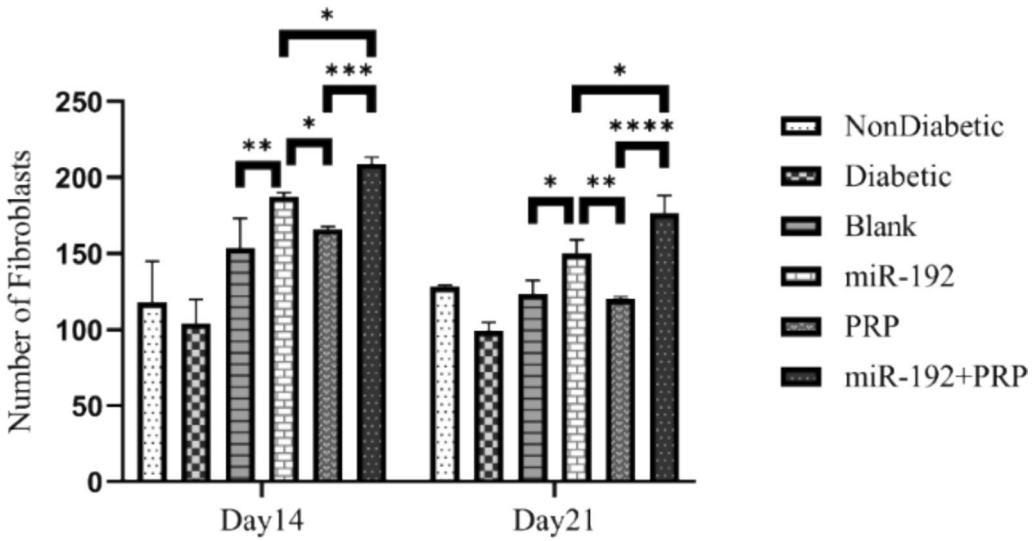


Fig. 10 Comparison of the average number of fibroblast cells on the 14th and 21st day after wounding between different intervention groups. NS (not significant) and * ($P < 0.05$) and ** ($P < 0.01$) and *** ($P < 0.001$) and **** ($P < 0.0001$)

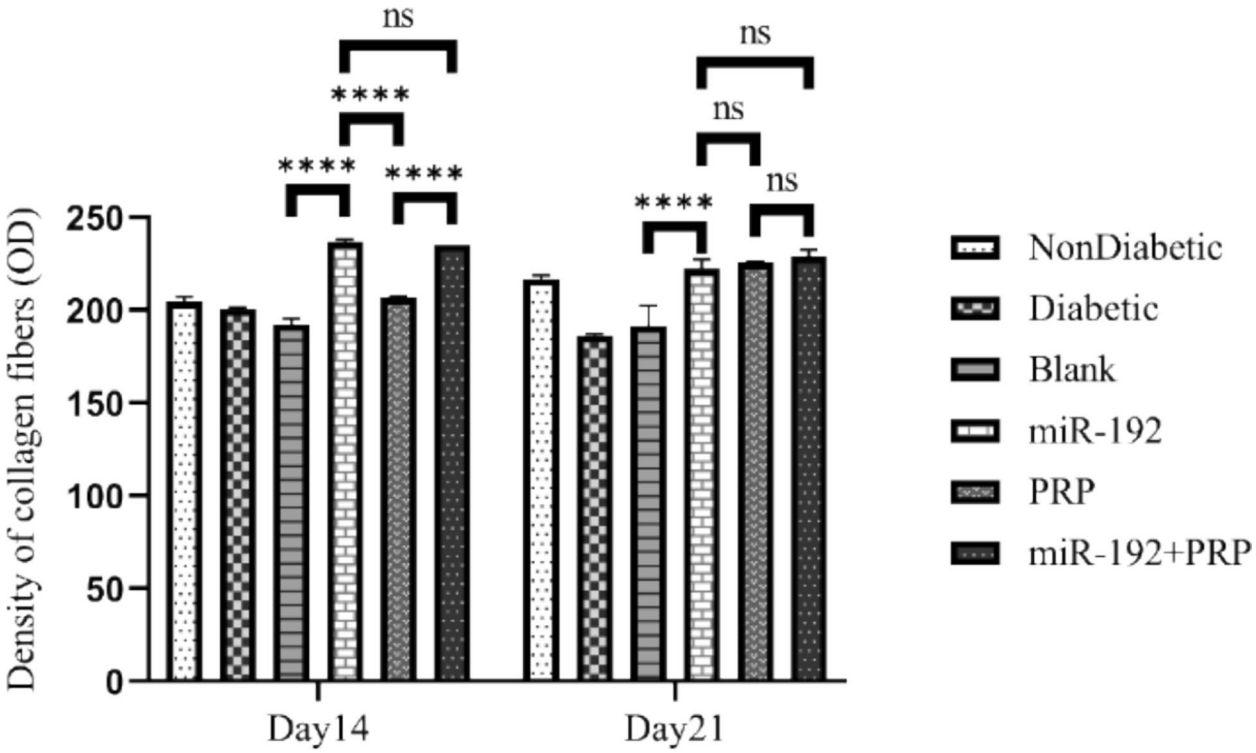


Fig. 11 Comparison of the average density of collagen fibers on the 14th and 21st days after wounding in different intervention groups. NS (not significant) and * ($P < 0.05$) and ** ($P < 0.01$) and *** ($P < 0.001$) and **** ($P < 0.0001$)

Combined treatment with PRP and HDF cells expressing miR-192 increased the area of granulation tissue and wound healing

On the seventh day, the group treated at the same time with PRP and HDF expressing pLenti-III-miR-192-GFP, the average area of the granulation tissue is 502188 μm square and more than other groups, and there is a critical difference between this group and the two groups treated with PRP and HDF containing pLenti-III-miR-192-GFP expression plasmids alone was seen $P < 0.0001$.

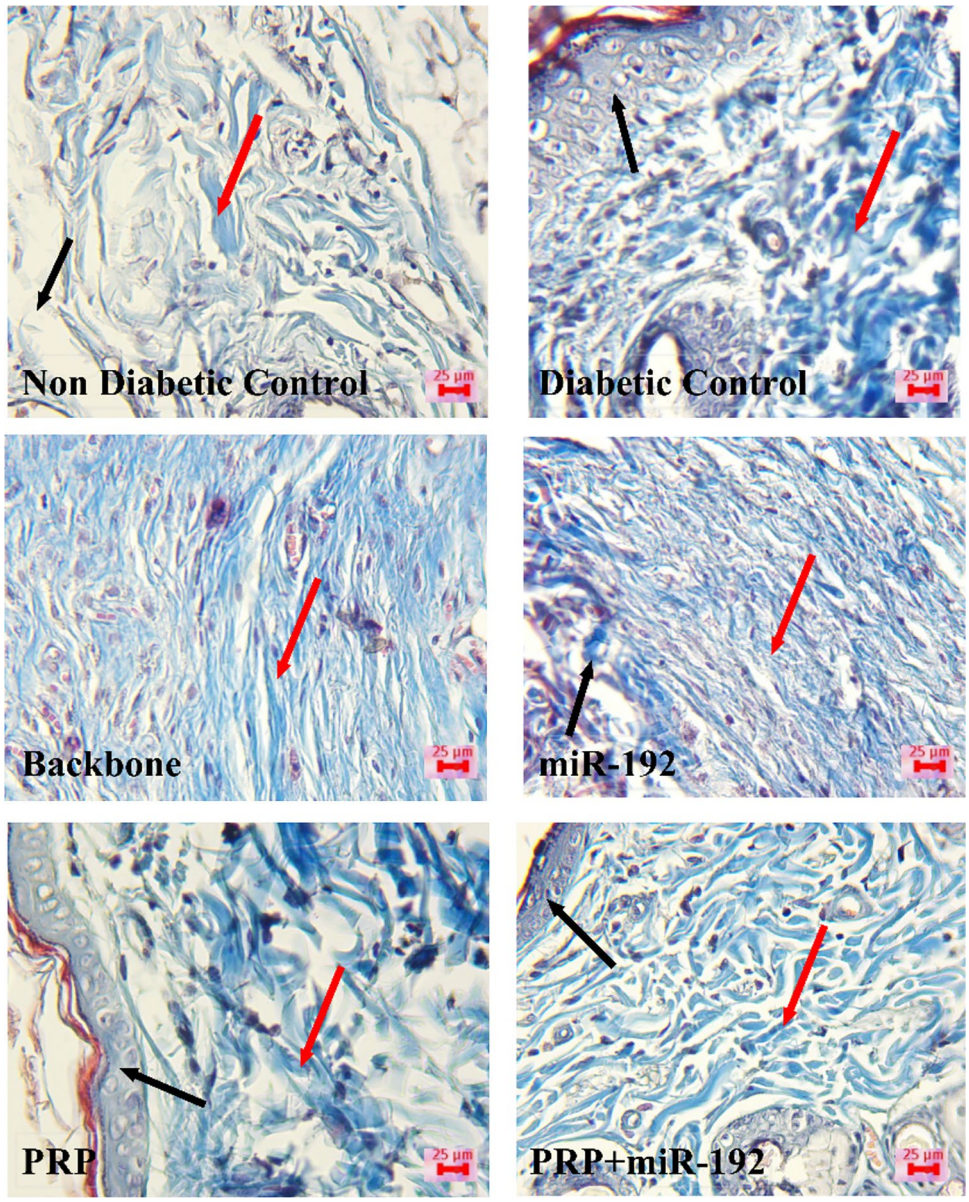


Fig. 12 A Masson-trichrome staining of wound area in different groups on day 14 after wound induction. Note: Black arrow: epidermis area. Red arrow: blue collagen fibers in the dermis. Photographed at a magnification of 400x, scale bar = 25 µm

On the 14th day, a noteworthy distinction was observed between the group that received the combined treatment and the group that was treated with a PRP $P < 0.05$. But there was no difference between the combined group and the group that had fibroblasts containing pLenti-III-miR-192-GFP. There was no noteworthy difference between the PRP group and the group treated with HDF expressing pLenti-III-miR-192-GFP. But in all the groups that received treatment, compared to the healthy and diabetic control groups, there was a critical distinction, and the area of the granulation tissue was greater within the intervention groups than within the control groups. (Fig. 13). The area of granulation tissue can be seen in hematoxylin–eosin staining in Fig. 14.

Discussion

Present-day medications for wound care are not completely successful and chronic wounds present a challenge within regenerative medicine. Gene therapy and genetic engineering are new approaches that appear to have helpful potential for treating persistent wounds. Bioactive particles such as DNA, mRNA, siRNA, and

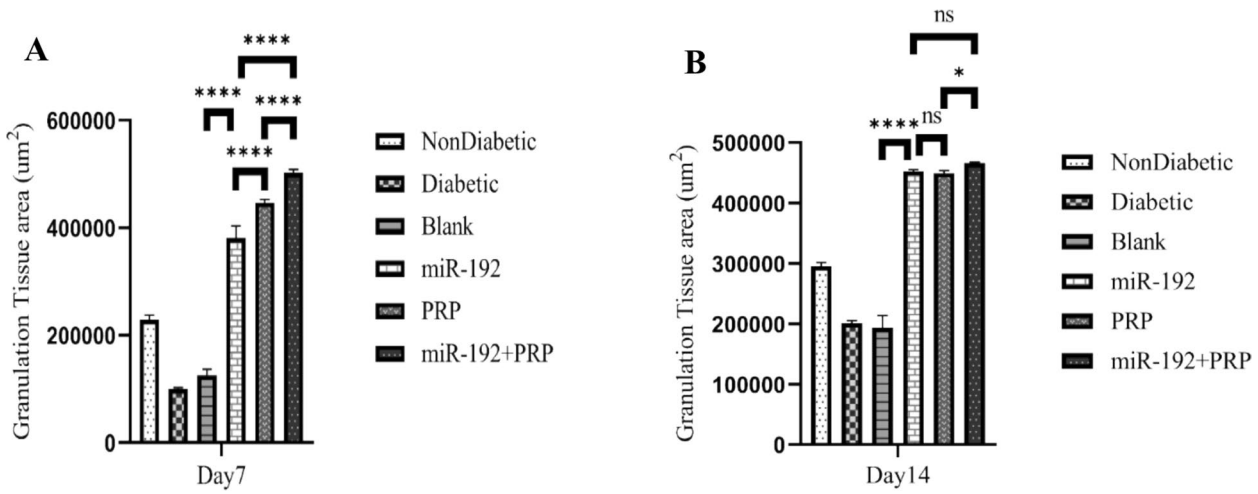


Fig. 13 Comparison of the area of granulation tissue on the seventh day and the 14th day after wound formation. **A** Comparison of the area of granulation tissue on the seventh day after wound formation in different intervention groups. **B** Comparison of the area of granulation tissue on the 14th day after wound formation in different intervention groups. NS (not significant) and *($P < 0.05$) and **($P < 0.01$) and ***($P < 0.001$) and ****$P < 0.0001$)

miRNA may offer incredible clinical advantages, especially in regulating complex genetic systems and cellular signaling cascades related to skin repair. Changed expression of miRNA has appeared to play a central part in adjusting protein expression, and miRNA-based interventions may offer a wide run of targets that can be directed by a single miRNA [23, 26]. In some studies, human skin fibroblast cells have been proposed as a capable helpful instrument for skin repair in wounds whose healing is delayed [27–29].

The present study used a genetic engineering approach to create more efficient fibroblast cells with increased expression of miR-192 and *COL1A2.* These modified cells were used in combination with platelet-rich plasma (PRP) for wound healing in an experimental model of diabetic rats. The results showed that increasing the expression of miR-192 in fibroblast cells resulted in increased expression of collagen, and the use of these modified cells locally in the wound area showed beneficial effects in accelerating the healing process of chronic wounds in the experimental model. Moreover, the combination treatment of PRP and fibroblast cells expressing miR-192 was found to be effective in promoting wound healing, suggesting a potential therapeutic application for this approach in the treatment of chronic wounds.

Li et al. [30] showed that in hypertrophic scars, increasing the expression of miR-192 by direct suppression of SIP1 protein within the TGF-/Smad2-3 signaling pathway enhances the expression of type 1 and 3 collagen and *α-SMA*.

According to Fang et al. [31] a cytoplasmic protein named SIP1 inhibits the phosphorylation of Smad2/3 and prevents the nuclear Smad from binding to the collagen gene promoter within the TGF-β/Smad2-3 signaling pathway and finally decreased the expression of collagen protein.

In our study, HDF cells transfected with pLenti-III-miR-192-GFP expression plasmids demonstrate significantly increased in the level of *COL1A2* expression compared to the group of HDF cells with pLenti-III-Backbone-GFP expression plasmids.

Increasing the content of collagen peptides in skin fibroblast cells in laboratory conditions increases the proliferation of fibroblast cells and the content of fibroblast-derived cell matrix [30]. Also the use of peptides derived from human *COL1A2* in laboratory conditions leads to an increase in collagen levels and cell migration and the amount of elastin in skin fibroblast cells [31]. In the present study, the results showed that the use of combined treatment of PRP cells and HDF cells expressing miR-192 supported the wound healing process and faster wound closure compared to both PRP and HDF cells expressing miR-192 alone, in addition, the group treated with fibroblasts expressing miR-192 showed a more critical increase in wound closure compared to the backbone group which may be due to the increased expression of miR-192 and increment in collagen expression in HDF cells. Consistent with our study, Zabihi et al. [32] examined the impact of injecting fibroblast cells on the healing of diabetic wounds in male rats, the speed of wound healing in conjunction with the thickness and elasticity of the skin increased altogether within the group treated

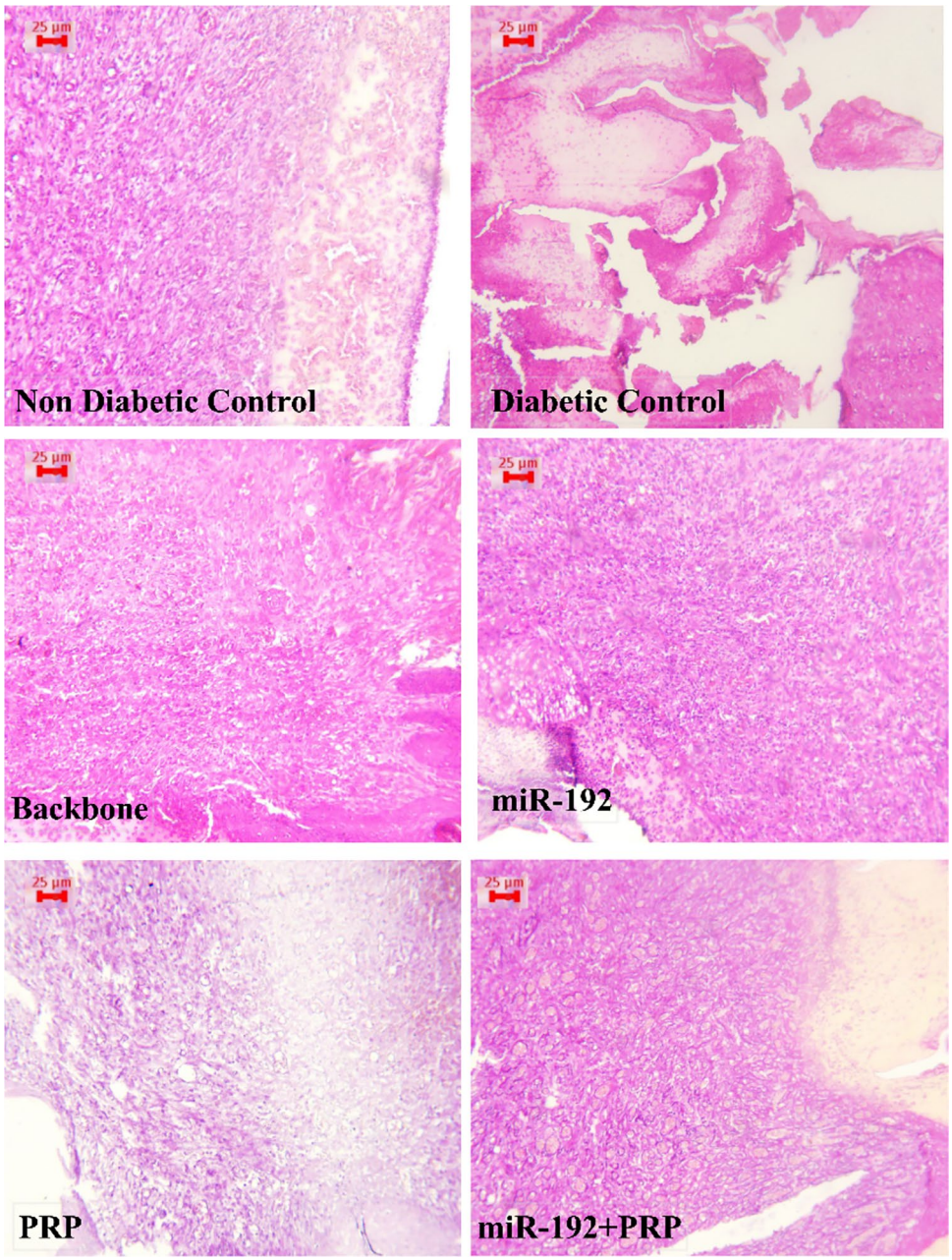


Fig. 14 Granulation tissue area in hematoxylin–eosin staining. H&E staining demonstrated the collagen fiber deposition and changes in capillaries in the granulation tissues photographed at a magnification of 40x, scale bar = 25 µm

with fibroblast cells, and it seems that fibroblast cells can accelerate healing by expanding the thickness and elasticity of the skin.

Mohammadipour et al. [33] found that platelet gel treatment significantly improved wound healing in rats by increasing collagen formation and inflammatory cell infiltration. Immunohistochemistry revealed higher vascular growth factor expression in the treatment group compared to the control group, in the current study, we showed that in the group that received the combined treatment of PRP and fibroblast cells containing miR-192, the amount of collagen and granulation tissue formation on days 7 and 14 after wound formation compared to the groups of PRP and fibroblasts expressing backbone was increased. Chuncharoni et al.[28] investigated the effect of platelet gel on skin wound healing in desert rats and used human platelets to heal skin wounds in rats, in their study, microscopic evaluation showed that on the 3rd and

7th days after wound formation, within the treatment group, the increase in the migration of epithelial cells and the arrangement of epithelial tissue at the wound site were well seen, the results of the present study are also consistent with the above findings, as our study used human platelets for diabetic skin wound repair. The present study is consistent with the findings of the above study, in our study, human platelets were used to repair diabetic skin wounds, and microscopic evaluations on days 7, 14, and 21 showed an increase in the movement of epithelial cells and the arrangement of the epithelium in the group treated with PRP cells and fibroblast expressed miR-192 compared to control groups. Shoaibi et al. [13] investigated the effect of platelet-rich plasma on the proliferation and migration of human skin fibroblasts. They showed that PRP stimulates the proliferation and migration of skin fibroblasts and also increases the expression of *procollagen 1 alpha*, *elastin*, *MMP1 and MMP2* in skin fibroblasts, thus, it can help accelerate healing. In the present study, the density of collagen fibers in the group treated with PRP and fibroblast cells expressing pLenti-III-miR-192-GFP was more than other groups on the 14th and 21st day after wounding, which indicates the effect of simultaneous use of PRP and fibroblast cells expressing pLenti-III-miR-192-GFP in the wound healing process. According to the data of the present study, the combination of PRP and fibroblasts expressing miR-192 was more effective than either alone and improved the wound healing process and its related characteristics, such as: wound closure rate, granulation tissue and the formation of epithelial tissue and angiogenesis and collagen fiber synthesis. In connection with our study Ni et al. [34] Combined PRP and adipose-derived stem cells (ADSC) to heal wounds in a mouse model, according to their study's findings, PRP and adipose-derived stem cells combined resulted in a better rate of wound healing than PRP and adipose-derived stem cells used alone, additionally the PRP + ADSC group had higher expression levels of *p-STAT3*, *VEGF*, and *SDF-1* than the other groups, moreover, combination therapy significantly increased the proliferation of endothelial cells. In future studies, the increased expression of collagen and proteins related to wound healing could be investigated more. Also, potential long-term outcomes of the combined treatment, such as scars or functional tissue repair, could be assessed with a follow-up of more than 21 days. This study could be followed by other studies investigating and evaluating combined treatments and gene therapy in regenerative medicine.

Conclusion

The percentage of wound closure, the number of fibroblast cells, the area of granulation tissue, the area of epithelial tissue, and the collagen fibers could all be significantly increased by using fibroblasts expressing miR-192, which increased the expression of collagen, additionally, this treatment was able to boost angiogenesis in the area of the diabetic wound, which aided in hastening the healing process in wound diabetic model.

Abbreviations

PRP	Platelet-rich plasma
DFU	Diabetic foot ulcer
COL1A2	Collagen type I alpha 2 chain
SIP1	Smad-interacting protein 1
HDF	Human dermal fibroblasts
GFP	Green fluorescent protein
DM	Diabetes mellitus
ECM	Extracellular matrix
PDGF	Platelet-derived growth factor
FGF	Fibroblast growth factor
TGF	Transforming growth factor
STZ	Streptozotocin

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Author contributions

FK, MS, and SD performed and analyzed most of the experiments and were significant contributors to writing the manuscript. All authors read and approved the final manuscript. Corresponding author Correspondence to Gholamreza Anani Sarab.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The ethics governing the use and conduct of experiments on animals were strictly observed, and the experimental protocol was approved by the Birjand University of Medical Sciences committee on Medical Research ethics (IR.BUMS.REC.1400.413).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran.

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