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Original Article

Human umbilical cord Wharton's jelly mesenchymal cell medium progress the wound healing via cytokines and growth factors expressions

Tahereh Ebrahimi[1], Vahideh Tarhriz[2], Haleh Forouhandeh[3], Fatemeh Sadat Shariati[4], Ali Sahraeian Jahromi[5], Mohammad Hosein Hadian Tabarestani[5], Hossein Fathollahzadeh[6], Masoud Delashoub[7*]

[1]Department of Nanobiotechnology, New Technologies Research Group, Pasteur Institute of Iran, Tehran, Iran; [2]Research Center for Infectious Diseases and Tropical Medicine, Tabriz University of Medical Sciences, Tabriz, Iran; [3]Molecular Medicine Research Center, Biomedicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran; [4]Infuenza Research Lab, Pasteur Institute, Tehran, Iran; [5]Graduate of Veterinary Medicine, Faculty of Veterinary Medicine, Tabriz Medical Sciences, Islamic Azad University, Tabriz, Iran; [6]Neurosciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; [7]Department of Basic Science, Faculty of Veterinary Medicine, Tabriz Medical Sciences, Islamic Azad University, Tabriz, Iran. [*]Corresponding author.

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Abstract: Objective: Conditioned medium of umbilical cord mesenchymal cells is a rich environment in various growth factors and cytokines, the use of which causes self-improvement and self-renewal in damaged tissues. Methods: Therefore, we investigated the effect of Wharton's umbilical cord mesenchymal cells on cytokines, growth factors expression, and skin wound healing in diabetic rats. Rats were divided into two groups of ten. In the treated diabetic group, 1 ml of conditioned medium was used intradermally, and in the diabetic control group, the same amount of physiological serum was used. The tissue samples were evaluated for histological studies. The expression level of inflammatory/anti-inflammatory cytokines and growth factors was investigated using RT-PCR and western blotting analysis. Results: Our results showed that wound healing increased in the diabetic rat group with a pleasant environment compared to the control group. It was also found in molecular studies that the expression of anti-inflammatory cytokines and growth factors was significantly increased in the treated samples compared to the control group. In addition, a significant decrease in TGF-β expression as an important inflammatory cytokine observed compared to the control group. Conclusions: The use of the conditioned environment of Wharton's jelly mesenchymal cells of the human umbilical cord improves the process of wound healing in terms of tissue and also increases the expression of the critical anti-inflammatory cytokines and growth factors. It can be considered a novel approach in wound healing treatment.

Keywords: Conditioned medium, mesenchymal cells, skin wound healing, cytokine, growth factor, diabetes

Introduction

Mesenchymal stem cells of the umbilical cord were isolated from the matrix of the umbilical cord for the first time in 2003. The cells can multiply indefinitely, and they differentiated into nerve and glial tissues [1]. Studies have shown that umbilical cord matrix stem (UCMS) cells can differentiate into nerve, muscle [2], cardiac [3], cartilage, and bone cells [4] in the culture medium, as well as injecting these cells into the brain rats have improved the symptoms of Parkinson's in rats and the differentiation of these cells into nerve cells [5]. Wharton's jelly of the umbilical cord contains connective tissue derived from extraembryonic mesoderm and fibroblast-like cells [6]. These cells express surface receptors CD44, CD105, internal markers CD51 and CD29, while human umbilical cord mesenchymal stem cells do not express hematopoietic markers CD45, CD14, CD33, CD34 and CD56 [7, 8]. The goals of tissue engineering are the reconstruction and regeneration of living tissues to replace defective or damaged tissues and organs to maintain, restore, or enhance their lost function [9].

Embryonic stem cells are a leading candidate for tissue engineering due to their high self-renewal capacity and pluripotency (the ability to differentiate into all germ layers) in the laboratory and *in vivo* [10]. Therefore, stem cells are the first hope for repairing damaged tissues and healing wounds [11]. Hemostasis, inflammation, reproduction and regeneration are the fourth stages of wound healing that overlap with each other. Any substance that can shorten the time of these stages leads to the acceleration of the healing process [12]. Wound healing is a complex, evolutionary, and multicellular process that aims to repair the epithelium after injury. These processes rely on a large number of growth factors and cytokines that activate and regulate a complex signaling network that alters the growth, differentiation, and metabolism of target cells. During acute wound healing, these biologically active polypeptides are present in the wound bed and play a role in all cases: inflammation, granulation tissue formation, matrix formation, re-epithelialization, and remodeling [13]. Proinflammatory cytokines, especially tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1 beta (IL-1 beta) IL-6, are upregulated during the inflammatory phase of wound healing. IL-1 cytokine is created by macrophages, monocytes, neutrophils, and keratinocytes cells. This cytokine is released by keratinocytes immediately after wound healing process. Furthermore, IL-1 activates fibroblasts and IL-6 that is important in initiating the healing response and produced by neutrophils and monocytes [14].

GFs regulate most of the processes involved in wound healing. The tissue repair process in wound healing is controlled by the interaction of growth factors with specific cell surface receptors. Epidermal Growth factors (EGF) and Vascular endothelial growth factor (VEGF-A) are among these factors. These factors stimulate the production of new cells, cell migration, stimulation of angiogenesis, and regeneration of the damaged area [15]. EGF which is secreted by platelets, macrophages and fibroblasts, plays an important role in epithelialization and collagen production in the damaged tissue [16]. EGF increases the proliferation of epithelial, endothelial, keratinocytes and fibroblasts, and also increase the process of angiogenesis, collagen production and increase of the thickness of the epithelium during the wound healing process [16]. Angiogenic factors like VEGF promotes angiogenesis through several mechanisms, including enhanced endothelial proliferation and migration [17, 18].

Wharton's jelly mesenchymal stem cells (WJ-MSCs) are multipotent stromal cells derived from the umbilical cord, widely recognized for their regenerative and immunomodulatory properties [19]. These cells exhibit a high proliferation rate, low immunogenicity, and multilineage differentiation potential, making them a promising tool for tissue engineering and regenerative medicine [20]. Unlike bone marrow-derived MSCs, WJ-MSCs are more accessible, ethically favorable, and show stronger paracrine effects, secreting a diverse array of cytokines, extracellular vesicles, and growth factors that promote wound healing [21]. Their secretome enhances fibroblast migration, keratinocyte proliferation, angiogenesis, and extracellular matrix remodeling, contributing to accelerated tissue repair. Furthermore, WJ-MSCs modulate inflammation by suppressing pro-inflammatory cytokines and promoting an anti-inflammatory environment, reducing fibrosis and improving functional recovery in damaged tissues [19]. Given their therapeutic potential, current studies are exploring their applications in diabetic wound healing, burns, and chronic ulcers, positioning WJ-MSCs as a cutting-edge approach in regenerative medicine [20, 22, 23].

In this current study, we aim to investigate the effect of Wharton's jelly mesenchymal cells of the umbilical cord, expressing the inflammatory/anti-inflammatory cytokines, expressing the GF genes, and skin wound healing in diabetic rats. Using the pleasant environment of mesenchymal cells of the umbilical cord, a rich environment in various cytokines causes self-improvement and self-renewal in damaged tissues.

Materials and methods

Preparation of animal samples and wound healing assays

In this experimental study, twenty adult male Wistar rats (20 ± 220 grams) were used. The animals were housed in the laboratory animal research and breeding center of Azad University, Tabriz under standard conditions (Research Ethics Committee approval ID: IR.

Table 1. Forward and reverse primers for quantitative RT-PCR analysis [27, 28]

Gene name	5'-3' primer sequence
TGF-β	F-CAAGGGCTACCATGCCAACT
	R-AGGGCCAGGACCTTGCTG
TNF-α	F-CCCCAGGGACCTCTCTCTAATC
	R-GGTTTGCTACAACATGGGCTACA
IFN-γ	F-GTTTTGGGTTCTCTTGGCTGTTA
	R-AAAAGAGTTCCATTATCCGCTACATC
IL-1	F-CCTGTCCTGCGTGTTGAAAGA
	R-GGGAACTGGGCAGACTCAAA
IL-6	F-AACCTGAACCTTCCAAAGATGG
	R-CTGGCTTGTTCCTCACTACT
IL-8	F-CATACTCCAAACCTTTCCACCCC
	R-TCAGCCCTCTTCAAAAACTTCTCCA
IL-10	F-GCTGGAGGACTTTAAGGGTTACCT
	R-CTTGATGTCTGGGTCTTGGTTCT
GAPDH	F-AGAAGGCTGGGGCTCATTTG
	R-AGGGGCCATCCACAGTCTTC

IAU.Tabriz.REC.1401.040). Diabetes was induced in these rats using streptozotocin (STZ) injection to create a diabetic model before the experimental procedures. Following confirmation of diabetes, the rats were randomly divided into two groups of ten, and a wound was created on their backs. In the first group, 1 ml of physiological serum was injected intradermally as a control. In the second group, 1 ml of mesenchymal cells derived from the umbilical cord was injected intradermally as the conditioning treatment group [24]. Thin sections (5-6 microns thick) of the skin tissues were prepared using standard tissue processing methods. These sections were stained with hematoxylin and eosin (H&E) to examine tissue regeneration and structural changes. The wound healing process was monitored over a period of 15 days. The wound surface area was photographed and measured using image-analysis program (ImageJ, version v1.54d) [25]. The percentage of wound healing was calculated by comparing the initial wound size to the remaining wound area on specific days. Statistical analysis was performed using SPSS software, with significance set at $P < 0.05$.

At the end of the experiment, all rats were humanely sacrificed following ethical guidelines for animal research. Euthanasia was performed using an overdose of ketamine (100 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally to ensure deep anesthesia. Once the absence of pain reflexes was confirmed, cervical dislocation was performed as a secondary method to ensure complete euthanasia. Following sacrifice, skin tissue samples were collected for histological and molecular analyses. Some tissue samples were fixed in a 10% formalin solution for histological analysis and other samples were quickly frozen for molecular studies.

RNA extraction and Real-Time PCR method (RT-PCR)

RNA was extracted from tissue samples, and the expression levels of inflammatory and anti-inflammatory cytokines were analyzed. The total RNA of samples was extracted according to the relevant conventional protocol [26]. To synthesize single-strand cDNAs 1 µl of random hexamer and oligo dT primers (SinaClon, Cat. No.: RT5201) with 1 µl of each RNA was added to 10 µl of Buffer-Mix (2×) and 2 µl Enzyme mix. The solution was poured into a 0.2 micro-tube and brought to a volume of 20 µl with DEPC-treated water. The micro-tubes were incubated for 1 min at 25°C, 60 min at 47°C, and 5 min at 85°C in a thermocycler, respectively. The RT-PCR method based on SYBR Green I fluorescence dye, which includes the introduction of free dye molecules between two newly synthesized DNA strands in each cycle was used. To perform the reaction, cDNA sample, 0.25 µl Forward primer, 0.25 µl Rivers primer (**Table 1**) [27, 28], and 3.5 µl dH_2O were added to 5 µl Master Mix (Ampliqon RealQ Plus2X master mix green, Denmark), then the microfuge was done at 2000 rpm for 20 seconds. In the next step, 1 µl of cDNA was added to the above mixture and transferred to the Real-Time device with a certain temperature and time program. The relative quantity of mRNA samples was detected using the standard $\Delta\Delta^{Ct}$ method [29, 30].

Quantitative western blotting tests

To confirm PCR results, TGF-β, IL-10, EGF and VEGF-A proteins level were evaluated in both treated and non-treated (control) samples. For this aim, the total protein of samples were isolated using a lysis buffer including 500 µl of Tris-Hcl, 3 mg of ethylenediaminetetraacetic acid (EDTA), 80 mg of NaCl, 25 mg of Deoxycholic acid sodium salt (DOS), 10 mg of sodium

dodecyl sulfate (SDS), and 10 µL of 1% Triton with protease inhibitory cocktail [31, 32]. The concentration of extracted proteins was monitored using the Bradford assayes. To isolate proteins, 12 µl of each protein sample was electrophoresed on 5% SDS-PAGE gel and replaced with the nitrocellulose membrane (PVDF) (Amersham, Cat. No: 10600023) using the semidry electrophoretic transfer method [31, 32]. A blocking solution was used to inhibit the non-specific reaction of the initial antibody. The primary antibody in the blocking solution was added to PVDF and incubated. Ultimately, the secondary antibody was utelized to detect mentioned protein band [29, 33]. The bands were visualized using chemoluminescence kit (ECL™, Cytiva, RPN418) [29, 33]. Anti-TGF-β antibody (sc-126, Santa Cruz Biotechnology), anti-IL-10 antibody (B-9: sc-7480, Santa Cruz Biotechnology), in a 1:500 dilution in PBS, 2.5% Blotto, 0.05% Tween-20; anti-GAPDH antibody (sc-365062, Santa Cruz Biotechnology) 1:10,000 dilution in PBS, 2.5% Blotto, 0.05% Tween-20, and mouse anti-rabbit IgG-HRP: (sc-2357, Santa Cruz Biotechnology) 1:10,000 dilution in PBS, 2.5% Blotto, 0.05% Tween-20 as secondary antibody was utilized [34].

Data analysis

Statistical analysis was performed using SPSS PASW Statistic 18 to evaluate differences between groups. The Student's t-test was used for inter-group comparisons, assessing variations in wound healing progression, cytokine expression, and protein levels. For intra-group comparisons over time, repeated measures ANOVA was applied to determine statistical significance across multiple time points. When comparing only two time points within the same group, a paired t-test was conducted. Additionally, Bivariate Correlation (Pearson/Spearman test) was used to analyze relationships between cytokine and growth factor expression levels and wound healing rates. All graphical representations were created using GraphPad PRISM Version 6.01. Statistical significance was set at $P < 0.05$.

Results

Examination of the wound surface

Wound healing is evaluated by measuring the wound surface, the healing percentage, and the time required for complete wound closure. The surface of the wound was measured by taking pictures perpendicular to the surface of the wound using image j software (**Figure 1A**). Appling a t-test, the average of wound surface was compared in two treatment and control groups. The results show that the average of wound level in the treated group has a significant decrease compared to the control group ($P < 0.05$) (**Figure 1B**). The percentage of wound healing was calculated for the studied days, and its values are shown in **Figure 1C**. The average percentage of wound healing per day is equal to the wound surface on the first day minus the wound surface on the day of the study divided by the surface of the wound on the first day. The results of examining the skin tissue slides in both the treatment and control groups showed that the epithelium in the treatment group was significantly thicker compared to the control group, which indicates the effect of the medium conditioning of mesenchymal cord cells on skin repair in diabetic rats.

RT-PCR analysis

To normalize the concentration of total RNAs, optical absorption ratios were measured using the spectrophotometric device (Thermo Fisher Scientific Inc - 5225 Verona Rd) in the wavelength (A^{260}/A^{280}). The expression level of the inflammatory cytokines/anti-inflammatory cytokines genes, and β-actin gene as an internal control was amplified using specific primers of each gene in a Real-Time PCR machine. Thus, the results of each sample were analyzed. Melting curve analysis was used to confirm the correctness of the amplified fragment and ensure the absence of non-specific product, primer dimer, and contamination. Analysis with SPSS software using a t-test showed that the expression of TGF-β, TGF-α, and IFN-γ in the samples of the treatment group decreased significantly compared to the samples of the control group (*P*-Value =0.040), while the expression of anti-inflammatory cytokines including IL-11, IL-7, IL-8, and IL-10 were significantly increased (**Figure 2**).

Analysis with SPSS software using a t-test showed that the expression of EGF and VEGF-A in the samples of the treatment group increased significantly compared to the samples of the control group (*P*-Value =0.040) (**Table 2**).

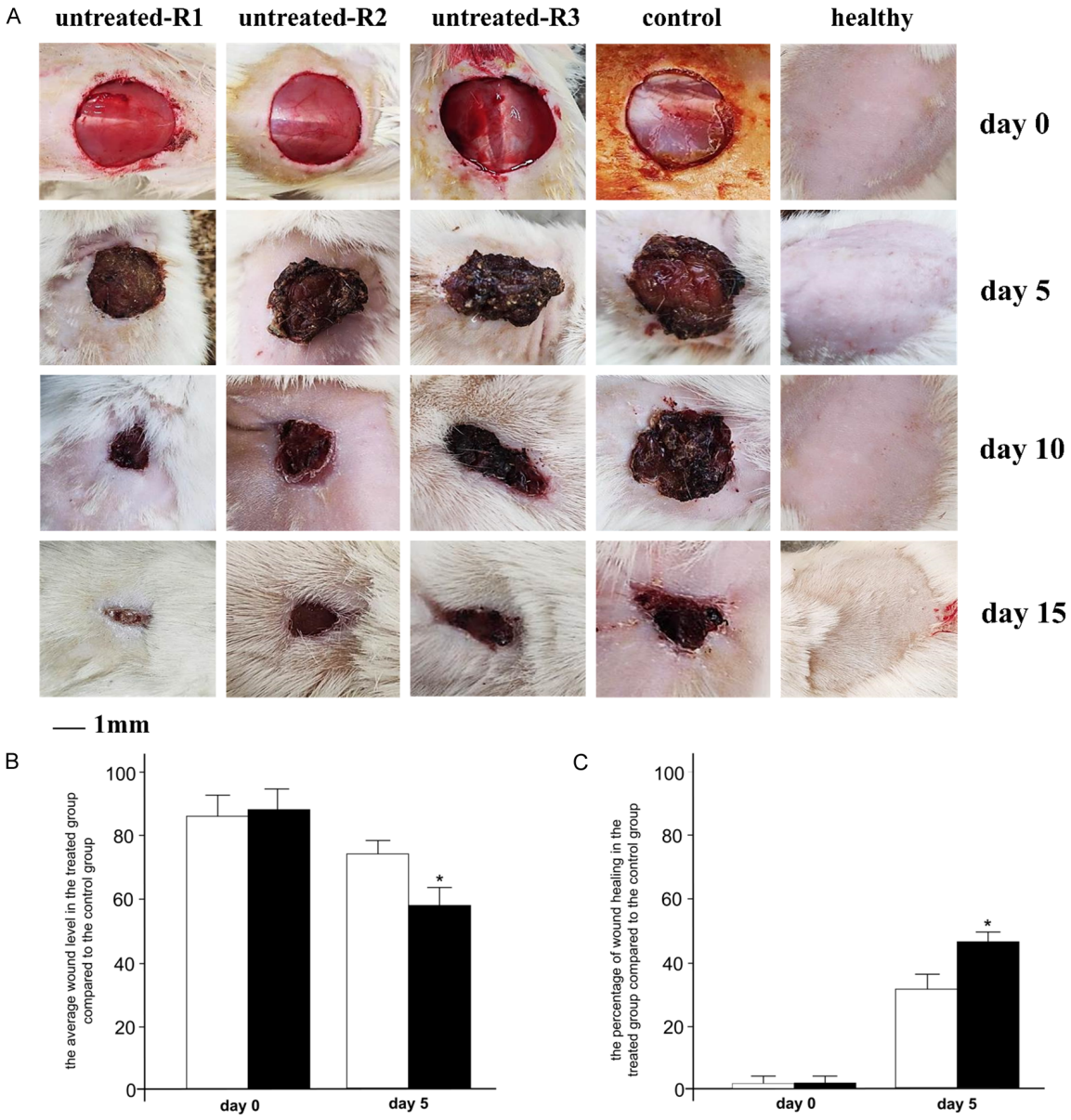

Figure 1. Wound healing process. A. Days 15 (from left to right) in the treatment groups R1 and R2 (R=repeat) and control group. B. The average wound level in the treated group compared to the control group ($P < 0.05$). C. The percentage of wound healing and its values in the treated group compared to the control group.

Western blotting results

To confirm the progression of the wound healing process by human umbilical cord wharton gel mesenchymal cell medium, we evaluated two critical inflammatory/anti-inflammatory cytokines including TGF-β and IL-10 protein expression levels. The data showed a significant decrease in TGF-β protein expression, while there was observed the upregulation of IL-10 protein expression level. The results of western blot analysis (**Figure 3**) are strong evidence to proof of RT-PCR results. These findings indicate the potential efficacy of the extract that is mediated by its ability to induce the progression of the wound healing process.

The results of examining the skin tissue slides in both groups showed the epithelial thickness was significantly different between the treated groups and the control group. The epithelial thickness increased in the conditioned medi-

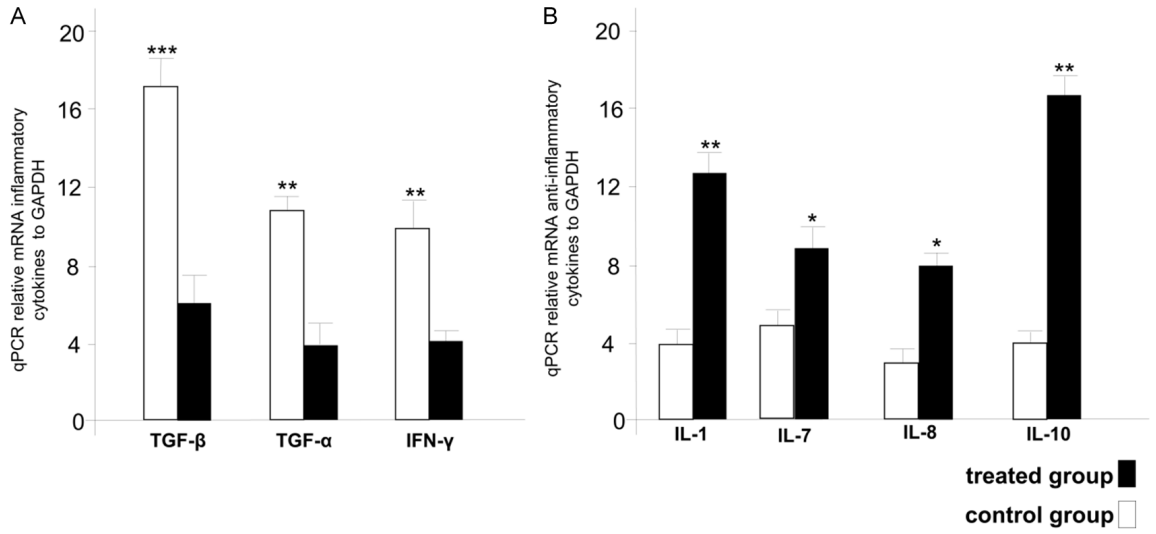

Figure 2. RTPCR analysis. Evaluation of (A) inflammatory cytokines including TGF-β, TGF-α, and IFN-γ, (B) anti-inflammatory cytokines including IL-1, IL-7, IL-8, and IL-10, *P < 0.05, **P < 0.01, and ***P < 0.001 indicate comparison between untreated (control) group and treated group.

Table 2. Comparison of mean expression of EGF and VEGF-A genes in control and treatment groups

Variation	Drug treatment Mean expression	Control Mean expression
VEGF-A	0.346 ± 0.06	0.114 ± 0.05
EGF	0.018 ± 0.005	0.007 ± 0.002

*P < 0.05 as compared to the control group.

um-treated group treated group which indicates the effect of the conditioned medium of umbilical cord mesenchymal stem on wound healing in diabetic rats' skin (**Figure 4**).

Discussion

According to global statistics, more than 270 million people are suffering from diabetes, and about 15% of these people suffer from wounds caused by diabetes. One of the serious complications of diabetes is the development of skin wounds, which in most cases become chronic, and take a long time to heal, and may even lead to amputation. The optimal repair of skin wounds depends on some complex biological and molecular processes such as cell migration, reproduction, extracellular matrix, angiogenesis and cell regeneration [35]. In diabetic wounds, the production of growth factors and extracellular matrix, as well as the process of angiogenesis, is greatly reduced, and this causes the wound to become chronic. The reduction of tissue regeneration and angiogenesis are factors that disrupt the healing process of diabetic wounds [36]. Other causes of delayed wound healing in diabetic patients include chronic wound inflammation, structural changes, thickening of the capillary basement membrane, decreased blood flow, and finally, high susceptibility to infection at the wound site [37]. Wound healing is a complex process including interactions between different types of growth factors, cells, and extracellular matrix compounds that depends on systemic signals such as chemokines, growth factors, cytokines, and proteolytic enzymes [38]. The use of new methods such as cell therapy as a non-invasive procedure can be a more suitable alternative to the old methods and patterns of treatment. Cell therapy is a scientific and effective application in the treatment of diseases and includes replacing cells in various injuries and damages [37].

In the current research, the effect of the human umbilical cord Wharton gel mesenchymal cell conditioning medium on the healing process of skin wounds in diabetic rats was investigated in the form of histological and molecular studies. The research showed the positive effects of using stem cells and their products, including the medium conditioning of stem cells in the healing process of skin wounds. At

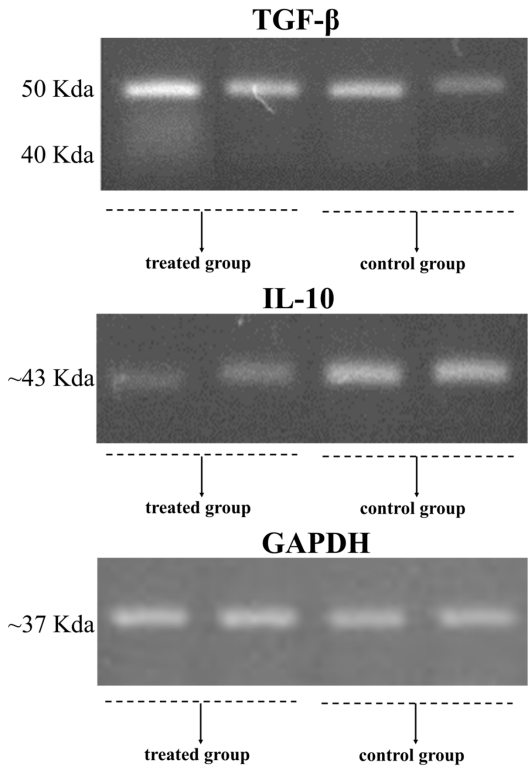

Figure 3. Western blot analysis. The expression level of TGF-β (50 and 40 Kd), IL-10 (between 40-50 Kd, ~43 Kd) and GAPDH (~37 Kd) Protein in treated group compared to untreated (control) cells.

a study by Wu et al. in 2007, the result showed that the injection of mesenchymal stem cells in the wound bed in diabetic rats had completed the process of new tissue formation on the 7th day after surgery. In addition, the treated wounds by mesenchymal cells have more capillaries in the wound bed [39]. Tarcisia et al. investigated the effects of the conditioning medium of fat mesenchymal cells on the proliferation phase in the clinical improvement of the wound healing process in rats, the results showed the rate and speed of epithelialization increased significantly [40]. In the study of Padta et al. in 2017, it was found that the use of stem cell medium conditioning cream is effective in healing wounds caused by burns in rats. Tissue studies conducted in this research show an increase in the number of fibroblast cells, the density of collagen fibers, and blood vessels. The medium condition includes various growth and tissue regenerating factors that are secreted by stem cells. In a other research, the use of different methods showed that it is possible to force the condition of the medium to make cells that help to treat diseases in the laboratory animal model [41]. The medium conditioning of mesenchymal stem cells could increase the migration of fibroblasts and keratinocytes in the damaged tissue. Furthermore, it was shown that growth factors could play a role in the migration of fibroblasts and cells in a conditioned medium release cytokines and extracellular matrix molecules. The treatment of skin wounds will be faster by conditioning the medium of mesenchymal stem cells. The mentioned cytokines are mostly for regulating the immune system, which helps to close the wound earlier [42]. In the present study, the effect of human umbilical cord mesenchymal cell Wharton gel medium conditioning on the inflammatory/anti-inflammatory cytokines expression in skin tissue during the wound healing process in diabetic rats was investigated. The statistical analysis of the results showed that the anti-inflammatory cytokines expression levels in the treatment group compared to the control group was a significant increase ($P < 0.01$). Similar studies with the present research in the field of expression of different cytokines in the wound healing process have given different results. Jayaraman et al. stated in 2013 that the use of a conditioned culture medium of stem cells in skin wounds could accelerate the wound healing process. This effect is due to having a wide range of cytokines and growth factors. Moreover, a conditioned culture medium can cause angiogenesis in the wound site, which indicates the presence of factors such as TGF-β [43]. The remodeling phase, during which collagen is synthesized, degraded, and dramatically reorganized (stabilized into a scar via molecular cross-linking), is also mediated by cytokines. Degradation of fibrillar collagen and other matrix proteins is performed by serine proteases and MMPs under the control of the cytokine network. MMPs not only degrade matrix components, but also act as regulatory molecules by directing enzymatic cascades and processing cytokines, matrix, and adhesion molecules to produce biologically active fragments [44]. Autocrine expression of TGF-beta 1 by leukocytes and fibroblasts, in turn, induces these cells to produce additional cytokines including TNF-α, IL-1 beta and PDGF, as well as Chemokines as components of a cytokine cascade [45]. Such factors act to perpetuate the inflammatory cellular response,

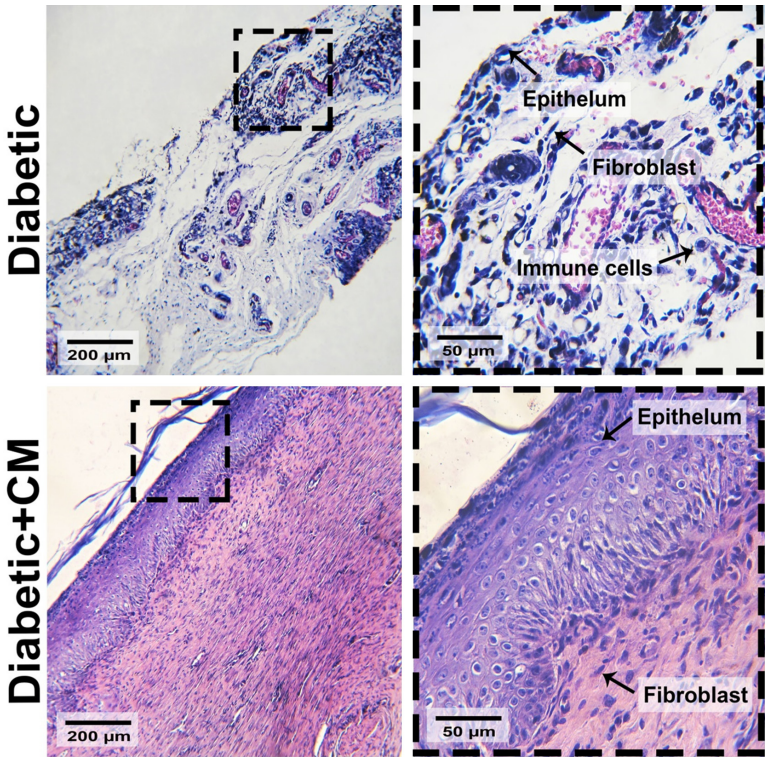

Figure 4. Micrograph image of wound healing in two conditioned medium-treated groups and the control group on day 14 with hematoxylin-eosin staining.

mediating the recruitment and activation of neutrophils and monocytes. As previously mentioned, pro-inflammatory cytokines, especially IL-1 and interleukin-6, and TNF-α are regulated in the inflammatory phase of wound healing. IL-1, which is released by keratinocytes after wound healing, in addition to having a paracrine effect, also increases the migration and proliferation of keratinocytes in an autocrine manner [14]. In addition, IL-1 activates fibroblasts and increases the secretion of FGF-7 [46]. IL-6 is critical in initiating the therapeutic response. Its expression increases after wounding and remains in older wounds [47-49]. It has mitogenic and proliferative effects on keratinocytes and chemotherapy for neutrophils [50-52]. Like IL-1, TNF-α can induce the production of FGF-7, suggesting that it can indirectly promote re-epithelialization [53, 54]. The effects of exogenous TNF-α are dependent on the concentration and duration of exposure, emphasizing the importance of the balance of proinflammatory signals that control wound healing. TNF-α, at low levels, can promote wound healing by indirectly stimulating inflammation and increasing macro-phage-produced growth factors [14].

Various types of growth factors play an essential role in the complex process of wound healing and tissue regeneration [55, 56]. EGF increases the rate of wound epithelialization and reduces scar formation thereby playing an important role in completing the wound healing process [57]. VEGF seems to be the strongest stimulation of angiogenesis during the wound-healing process. In the present study, the effect of conditioned medium derived from human umbilical cord Wharton's jelly mesenchymal stem cells (UC-MSCs) on GFs gene expression during the wound healing of skin cells in diabetic rats was investigated. The statistical data analysis showed that the GFs gene expression in the treatment group compared to the control group was a significantly higher increase ($P < 0.05$). Similar studies with the current research in the field of expression of different GF genes involved in the wound healing process have given different results.

The results of Wu et al.'s study showed that the injection of mesenchymal stem cells in diabetic rats leads to angiogenesis and a faster healing process. MSCs increase the expression of VEGF-a and Ang-1 factors, which play an important role in angiogenesis in the wound bed [39]. Jayaraman et al. stated that the use of stem cells conditioned medium in skin wounds can accelerate the wound-healing process [43]. This effect is due to a wide range of growth factors and cytokines which have a significant clinical impact on wounds. Also, a conditioned culture medium promotes angiogenesis in the wound site using several specific pro-angiogenic factors such as EGF, FGF, KGF and VEGF-A [43].

De Masi et al. studied the influence of growth factors on the healing process in rats. The

results of their study indicated that a combination of factors (FGF, VEGF and IGF) led to faster healing compared to other groups that received an injection of EGF. The macroscopic results revealed that the use of growth factors, both EGF and a combination of growth factors accelerates healing, stimulates greater angiogenic activity, and accelerates fibroplasia and collagen maturation [58].

As a limitation, our study is a lack of full understanding of growth factors regulation attributing to wound healing. Future studies would be needed to fully elicit the potential effect of MSC-CM on wound healing acceleration.

Conclusion

This study highlights the significant potential of conditioned medium derived from Wharton's jelly mesenchymal stem cells (WJ-MSCs) in promoting wound healing in diabetic rats. The findings demonstrate that the application of WJ-MSC-conditioned medium enhances tissue repair, modulates inflammatory and anti-inflammatory cytokine expression, and upregulates key growth factors essential for regeneration. Histological and molecular analyses confirmed improved epithelial thickness, reduced inflammation, and increased expression of pro-healing factors in treated groups. The statistical analysis further supports these results, indicating significant differences between treated and control groups in terms of wound closure rates and cytokine/growth factor expression. Given the challenges associated with chronic wounds in diabetic patients, WJ-MSC-conditioned medium presents a promising, non-invasive therapeutic approach for accelerating wound healing and tissue regeneration. Future studies should explore the long-term effects, optimal dosage, and potential clinical applications of this treatment in human subjects. The integration of stem cell-derived therapies into regenerative medicine could provide novel solutions for chronic wound management, improving patient outcomes and quality of life.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Masoud Delashoub, Department of Basic Science, Faculty of Veterinary Medicine, Tabriz Medical Sciences, Islamic Azad University, Tabriz, Iran. E-mail: masouddelashoub1976@iau.ac.ir

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