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Adipose Derived Stem Cell-Conditioned Media As Alternative of Stem Cell Therapy for Diabetic Wound Healing

Ayesha Nawaz[1], Sania Akram[1], Hazrat Zaman[1], Muhammad Shahbaz Aslam[2], Zaigham Abbas[1]*

1. Institute of Microbiology and Molecular Genetics, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590. Pakistan
2. Institute of Biochemistry and Biotechnology, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590. Pakistan

Corresponding Author's Email: zaigham.mmg@pu.edu.pk

ABSTRACT: *Diabetes mellitus (DM) is a set of metabolic disorder primarily characterized by chronic hyperglycaemia state, which is linked with several complications including retinopathy, neuropathy, and delayed wound healing. Adipose derived stem cells conditioned media (ADSCs-CM) contains several cytokines, and growth factors that can be used for treatment of diabetes-associated wounds. This study was conducted to explore the therapeutic potential of ADSCs-CM in excisional wound model in diabetic mice. Diabetes was induced in mice through Streptozotocin (STZ). Then, excisional wound splinting model in diabetic mice was developed. Subcutaneous injections of ADSC-CM (pure) and ADSC-CM conjugated with zinc sulphate were applied on the mice wound bed. Wound diameter, weight, and blood glucose levels of all the mice groups were measured at the start and end of the experiment. Additionally, qPCR was performed to analyse the expression level of some crucial genes (MMP-2, NANOG and OCT4) associated with wound healing. Overall, the results showed reduced wound size, blood glucose level, and weight restoration in ADSCs-CM treated mice as compared with control groups. Furthermore, ADSCs-CM also modulated the gene expression of MMP-2, NANOG, and OCT4, contributing to the enhanced wound healing observed in diabetic mice. This suggests ADSC-CM's potential as an alternative therapeutic option for achieving improved wound healing outcomes in diabetic patients.*

Keyword: ADSC-CM, Diabetics, Wounds healing, Blood glucose level

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder primarily characterized by hyperglycemia (high blood sugar) resulting from defects in insulin action, secretion, or both (Kharroubi and Darwish, 2015). Insulin, a vital hormone consists of 51 amino acids, plays a significant role in metabolism, cell growth, and glucose homeostasis (Lewis and Brubaker, 2021). It is a chronic and complex disease that has rapidly increased on a global scale (Hernandez et al., 2019). The International Diabetes Federation (IDF) predicts a substantial rise in the worldwide number of adults affected by diabetes, projected to increase from 422 million in 2017 to 642 million in 2040 (Ogurtsova et al., 2017). The hyperglycaemic state in diabetes can lead to severe complications, including peripheral neuropathy, stroke, diabetic kidney disease, and cardiovascular diseases (Fowler, 2008).

A major problem faced by diabetic patients is delayed or impaired wound healing, often resulting in chronic ulcers. The exact cause of delayed wound healing in diabetes remains unclear. However, research suggests a complex interplay between disease-specific intrinsic factors such as blood supply issues, increased matrix progression, abnormal angiogenesis, and extrinsic factors like infections and continued trauma (Spampinato et al., 2020). Furthermore, impaired cytokine production by fibroblasts and inflammatory cells also contributes to non-healing wounds in diabetic patients. Diabetic foot ulcers present a significant socio-economic and clinical burden, with an annual prevalence among diabetic individuals ranging between 1% to 4.1%. The incidence of amputations in this group is reported to be between 0.21% to 1.37%. Shockingly, the frequency of amputations in diabetic individuals is 15 to 70 times higher than that in the general population (Lin et al., 2016). The optimal healing of cutaneous wounds requires the integration of several molecular and biological events, such as cell proliferation, migration, angiogenesis, and extracellular matrix deposition and remodeling. In diabetes, this orderly sequence of events becomes disorganized, leading to compromised wound healing (Lux, 2022). Varoius treatment modalities are utilized to control diabetic wounds, including negative pressure dressings, bioengineered skin transplants, growth factor therapies, chemical debridement techniques, and hyperbaric oxygen treatment. However, these methods have limitations, such as providing only short-term relief and potentially inducing side effects (Game et al.,

2012). Consequently, diabetic wounds persist as a significant socio-economic challenge, necessitating a multidisciplinary approach to address these shortcomings in wound healing among diabetic patients. In recent times, cell-based therapy has garnered attention for treating multiple impaired wounds in diabetic patients. Stem cells in regenerative medicine possess the ability to self-renew and differentiate into various cell types (DiMarino et al., 2013). Previous reports indicate that adipose tissue-derived stem cells (ASCs) and bone marrow-derived mesenchymal stem cells (BM-MSCs) can promote wound healing in experimental diabetic models. Studies have shown that adipose-derived stem cells (ADSCs) secrete multiple growth factors, such as basic fibroblast growth factor (BFGF), hematopoietic growth factor (HGF), and vascular endothelial growth factor (VEGF), in the wound tissue, thereby enhancing angiogenesis and promoting refractory wound healing in both normal and diabetic mice (Zheng et al., 2020). Furthermore, stem cell-derived conditioned medium (SC-CM) has emerged as an effective tool for treating chronic wound healing. This medium contains essential cytokines necessary for healing chronic wounds, particularly diabetic wounds that are slow to heal and often persistent (Lupu-Haber et al., 2019). Consequently, SC-CM presents an alternative approach to overcome the limitations associated with cell-based therapies in chronic wound healing. Thus, this study was designed to assess the effectiveness of adipose-derived stem cell conditioned medium (ADSCs-CM) and ADSCs-CM combined with Zinc Sulfate as a potential alternative therapies for treating diabetic wounds in a mouse model.

MATERIALS AND METHODS

Experimental animals

For this study, a prior approval was taken from the Institutional Biosafety and Ethical Committee of Microbiology and Molecular Genetics, University of the Punjab, Pakistan. All experiments were carried on BALB/C mice (8 weeks of age, 20 ± 5g) and were kept at constant temperature (24 ± 2°C) in natural light–dark cycle (12–12 hrs) in the laboratory animal house of the Institute. All mice were fed with standard diet and water *ad libitum.*

Diabetic Induction in Mice

Diabetes was induced in mice according to the established protocols with a single intra peritoneal injection of streptozotocin (STZ) (150mg/kg Sigma-Aldrich, St. Louis, Mo). After the induction of diabetes in mice, 10% glucose water was given for 3 days to boost its blood glucose level and was

replaced with normal water on 4th day. For confirmation of diabetes in mice, blood glucose level of mice was measured after 7 days of STZ injection. Mice which had glucose level around 400mg/dL were selected for diabetic study (Dewey, 2014).

Animal Model

All animals were treated humanely according to the standard protocols and guidelines provided by the National Institute of Health (NIH). Mice were anesthetized by Intraperitoneal (I.P.) injection of 50µL ketamine, 12.5 µlxylazine and 187 µl of distilled water/mouse in 1mL (U-100,30 G×8mm) syringes. Hairs on the dorsal side of the mice were removed using an electric shaver then a depilatory agent was applied for complete removal of it. For injury induction, a previously similar protocol was followed with minor modifications. Mice were positioned dorsal side up on a sterile sheet. A wound of 5 mm in diameter was created (Chen et al., 2021; Alrouji et al., 2023).

Experimental Design and Treatment of Wounds

Mice were randomly divided into 4 groups, each containing 5 animals (n = 5), to evaluate the role of ADSCs-CM and ADSCs-CM conjugated with zinc sulfate in diabetic wound healing. Group I comprised healthy controls without diabetes, receiving no treatment after the induction of dorsal skin wounds. Group II, the diabetic control group, had skin wounds induced in mice but did not receive treatment with either ADSCs-CM or ADSCs-CM + zinc sulfate. Group III received 100 µL of ADSCs-CM, while Group IV received 100 µL of ADSCs-CM conjugated with zinc sulfate, administered at two sites in the dermis surrounding the wound using a sterile applicator (Joseph et al., 2020).

Preparation of Adipose Derived Stem Cells Conditioned Medium (ADSCs-CM) and its Conjugation with Zinc Sulfate

The mice underwent disinfection using 70% ethanol, and subcutaneous adipose tissue (300-600 mg) was collected from non-diabetic mice. The tissue was then cut into small pieces, placed in a petri plate containing phosphate-buffered saline (PBS) with 3% penicillin-streptomycin, and washed with PBS four additional times. Subsequently, the tissues were digested using collagenase-I solution (1mg/ml Millipore SCR103) in a shaking incubator for one hour. At the end of the incubation, a clear fat solution was obtained, which was centrifuged at 4000rpm for 20 minutes to collect the somatic vascular fraction (SVF) pellet containing adipose-derived stem cells (ADSCs).

The pellet cells were resuspended in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum, 1% L-Glutamine, and 1% Pen/Strep. Upon reaching 80% confluence at passage four, the spent media was replaced with fresh basal DMEM and incubated for three days in a 5% CO_2 environment. After three days, the conditioned media were collected, filtered using a 0.22µm filter, vacuum-sealed, and stored at 4ºC for future therapeutic applications (Ishida et al., 2020; Joseph et al., 2020).

Half of the ADSCs-CM was conjugated with 3% Zinc sulphate in final concentration by thoroughly mixing zinc sulfate particles into the ADSCs-CM until fully suspended. The study then evaluated the wound healing properties of pure ADSCs-CM and ADSCs-CM in combination with zinc sulfate.

Determination of Body Weight and Blood Glucose Levels

The weight and blood glucose levels of all experimental mice were measured on day 0, the starting day, and on day 14, the final day of the study. Blood glucose levels were measured using a Glucometer (ACCU CHEK Active) for all experimental groups.

Estimation of Wound Healing Closure

Wound healing was assessed using a vernier calliper on days 0, 3rd, 7th, and 10th after surgery by the same experimenter to minimize potential errors. The percentage of wound closure was calculated using the formula: Area of original wound = 1 - (Area of actual wound / Area of original wound) × 100, represented as (1 - A1/A0) × 100 (Chen et al., 2019).

RNA Isolation and Quantitative PCR (qPCR) Analysis

The skin samples surrounding the wound were collected, and total RNA was isolated using the Trizol method (Invitrogen, USA) (Than et al., 2015). The Thermo Scientific RevertAid First Strand cDNA Synthesis Kit was utilized to perform reverse transcription of the isolated RNA into cDNA following the manufacturer's protocol (Thermo Scientific, USA). Quantitative real-time PCR (qPCR) analysis was conducted to assess the expression levels of *MMP-2*, *NANOG*, and *OCT4* before and after the healing process using Maxima SYBER Green/ROX qPCR Master Mix 2x (Thermo Scientific, USA) (Than et al., 2015).

The qPCR reaction involved an initial denaturation at 95ºC for 10 minutes, followed by 45 cycles of amplification at 95 ºC for 10 seconds, annealing, and extension simultaneously for 30 seconds. The reactions were allowed to cool for 5 minutes. Table 1 contains the primer sequences for each marker. The

Delta Ct method was employed to calculate changes in gene expression.

Table 1:*GAPDH, NANOG, OCT4,* and *MMP-2*, primer sequence used for qPCR

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
GAPDH	TGACCTCAACTACATGGTCTACA	CTTCCCATTCTCGGCCTTG
NANOG	CACAGTTTGCCTAGTTCTGAGG	GCAAGAATAGTTCTCGGGATGAA
OCT4	CGGAAGAGAAAGCGAACTAGC	ATTGGCGATGTGAGTGATCTG
MMP-2	CCCTGGTGGCTGGAGGCTCT	AACGGGGTCCCACGTCCCAA

Statistical Analysis

Statistical analyses were conducted using Graph Pad Prism version 6 software. Arithmetic means and Standard error of all parameters were calculated, represented as error bars and ± signs on the graphs and tables, respectively. To assess the data, one-way ANOVA and t-tests were applied. The difference $p < 0.05$ were considered statistically significant.

RESULTS

Body Weight

The induction of diabetes in mice using Streptozotocin initially led to a decrease in weight due to its targeting of insulin-producing beta cells. Throughout the two-week study period, the body weight remained relatively stable in the ADSCs-CM group (18-20 g) and the ADSCs-CM plus zinc sulfate group (19-20 g) while in the diabetic control group, there was a significant drop from 14-20g. Additionally, weight loss consistently improved in both treated groups, whereas it decreased in the diabetic control group, as illustrated in Fig. 1.

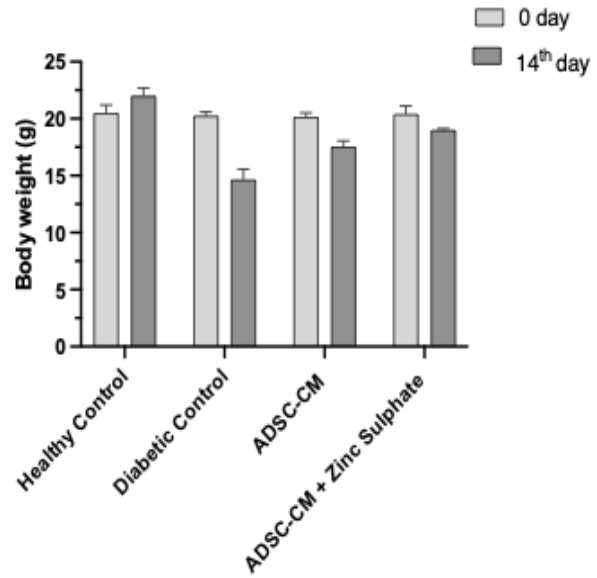

Fig 1. Effects of ADSCs-CM on body weight gain in diabetic mice.

ADSCs-CM Treatment Reduced Blood Glucose Levels

Hyperglycemia is primary concern for individuals with diabetes. In our study, the healthy control group mice maintained a consistent blood glucose level of 100 mg/dL. Conversely, the mice in the diabetic control group exhibited the high blood glucose level at 400 ± 50 mg/dL. This confirmed the adverse impact of Streptozotocin

administration on the efficiency of beta pancreatic cells, resulting in elevated blood glucose levels.

Furthermore, the group of mice treated with ADSCs-CM + zinc sulfate and ADSCs-CM demonstrated significantly decreased blood glucose levels of 200 mg/dL and 250 mg/dL, respectively, compared to the diabetic control group (refer to Fig. 2). Notably, the ADSCs-CM + zinc sulfate treated group effectively controlled blood glucose levels better than all other groups.

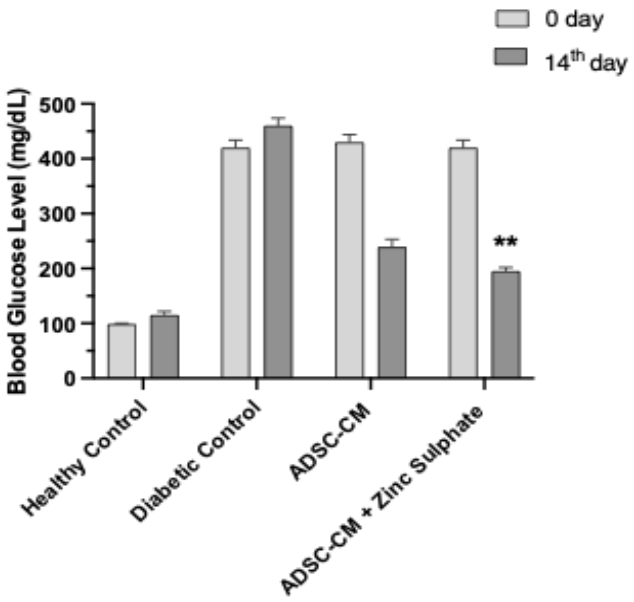

Fig. 2. ADSCs-CM caused reduction in blood glucose level of *streptozotocin* (STZ) induced diabetic mice.

ADSCs-CM Enhanced Diabetic Wound Healing

Overall, our study revealed that the transplantation of ADSCs-CM and ADSCs-CM conjugated with zinc sulfate in diabetic mice enhanced the wound healing process compared to the control groups. Substantial wound closure was noticeable as early as the 3rd day post-surgery and became more prominent by the 7th day in the treated mice groups compared to the diabetic control group. Additionally, a significant increase in wound closure was observed by the 10th day post-surgery in the ADSCs-CM conjugated with zinc sulfate (2 mm) and ADSCs-CM (2.8 mm) groups, while delayed wound healing was evident in the diabetic control (4.6 mm) and healthy control (4.4 mm) groups, as depicted in Fig. 3A.

Statistical analysis of wound diameter on days 0, 3, 7, and 10 further supported these observations across all treated and control groups, as illustrated in Fig. 3B.

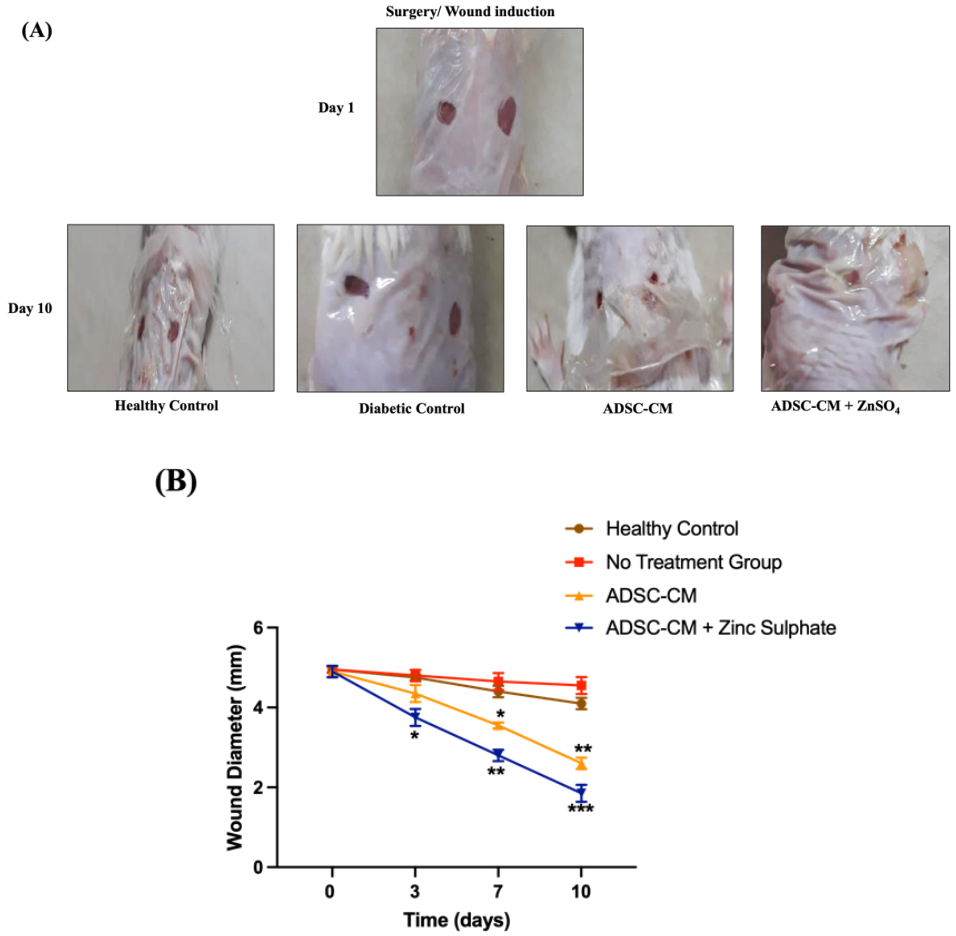

Fig. 3. Enhanced wound closure in diabetic mice treated with ADSCs-CM

ADSCs-CM Treatment Regulate the Expression of *MMP-2, NANOG* and *OCT4* Genes

To delve deeper into the effects of ADSCs-CM treatments on wound healing at the molecular level, we analyzed the mRNA expression of *MMP-2*, a pivotal enzyme in wound healing, along with *NANOG* and *OCT4* genes, crucial for cell proliferation and self-renewal, using qPCR. The results revealed a reduction in *MMP-2* expression, coupled with increased expression of *NANOG* and *OCT4* genes following ADSCs-CM treatment. These changes suggest accelerated healing in the treatment groups compared to the control groups, as illustrated in Figure 4. *GAPDH* served as the housekeeping gene for normalization.

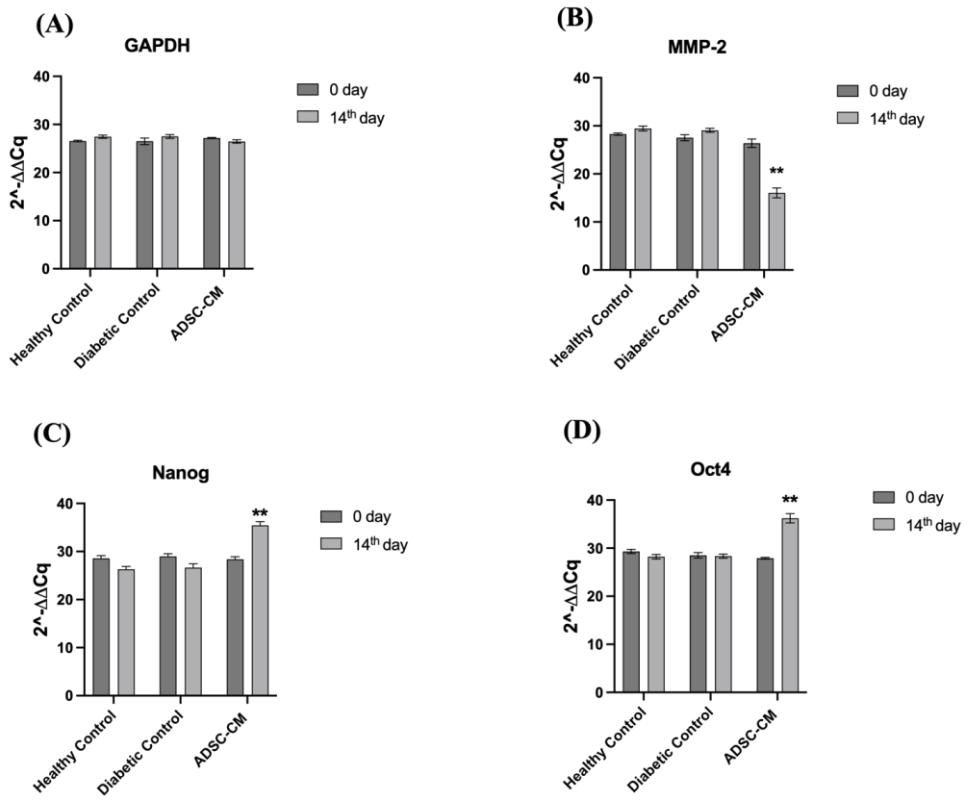

Fig. 4. Relative expression *MMP-2, NANOG,* and *OCT4*

Data is presented as mean ± SD. Number of samples (n) = 5. Experimental values are considered significant when p value is < 0.05. (*: p<0.05, **: p<0.01,).

This study strongly indicates that ADSCs-CM promotes the wound healing process in diabetic mice by modulating the expression of several key genes directly associated with wound healing, including *MMP-2*, *NANOG*, and *OCT4.* Consequently, the findings emphasize the significant potential of ADSCs-CM treatment as an alternative therapeutic option for diabetic wound healing.

DISCUSSION

Diabetes mellitus occurs when the body is unable to secrete or respond to insulin, resulting in elevated blood glucose levels in individuals (Olisah et al., 2022). Medically, it is associated with the long-term impairment of vital organs such as the heart, kidneys, nerves, eyes, and blood vessels. Additionally, it poses a life-threatening risk for both the unborn child and the mother, affecting 7% of total pregnancies annually (Cheng et al., 2008). By 2045, it is estimated that approximately 48% of the world's population will be affected by diabetes (Standl et al., 2019)

Furthermore, among the various

complications associated with diabetes, impaired wound healing emerges as a major clinical issue (Davis et al., 2018). This impairment is attributed to multiple physiological factors and deficiencies prevalent in diabetic patients, These factors encompass impaired angiogenesis, reduced production of growth factors, defective macrophage function, collagen accumulation, quantity of granulation tissues, compromised fibroblast and keratinocyte proliferation and migration, hindered epidermal barrier function, bone healing, epidermal nerve count, imbalance in extracellular matrix (ECM) accumulation and their remodelling by matrix metalloproteinases (MMPs), nuclear localization of ß-catenin, c-myc over expression, diminished and aberrant localization of estimated glomerular filtration rate (EGFR), and inactivation of the glucocorticoid pathway (Qing 2017).

To address this challenge, advancements in conventional methods need to be complemented by new alternative therapeutic measures (Menke et al., 2008; Szunerits et al., 2021). Therefore, there is a pressing need to explore novel and effective therapeutic approaches to enhance the diabetic healing process. Previously, mesenchymal stem cells (MSCs) have shown promise in enhancing diabetic wound healing due to their ability to migrate to the injury site, stimulate resident progenitors for proliferation and differentiation, secrete growth factors, and induce anti-inflammatory and immunomodulatory effects (Athanassiou et al., 2020). Over the last two decades, adipose-derived stem cells (ADSCs) have gained preference in regenerative medicine due to their rapid in-vitro proliferation, easy accessibility, low immunogenicity, and suitability for autologous transplantation (Athanassiou et al., 2020). However, the clinical applications of stem cells are constrained by safety concerns and issues related to cell viability (Mazini et al., 2020). Hence, significant interest has recently shifted toward cell-free treatments like ADSCs-CM and extracellular vesicles (EVs). ADSCs-CM contains various proteins (such as growth factors and cytokines), lipids (e.g., prostaglandins), and nucleic acids (non-coding RNA) that promote wound healing (Guo et al., 2022). Therefore, this study was conducted to evaluate the role of ADSCs-CM and ADSCs-CM combined with zinc sulfate in treating diabetic wounds and assessing their impact on blood glucose levels and associated molecular markers. Overall, our study demonstrates promising results, suggesting ADSCs-CM as a viable alternative therapy for diabetic wounds.

At the start of our study, induction of diabetes using streptozotocin resulted in

decreased body weight and increased blood glucose levels in mice. Streptozotocin, known for its high toxicity, contributes to the destruction of pancreatic beta cells, consequently leading to elevated blood glucose levels (Dewangan et al., 2017). However, following treatment with ADSCs-CM and ADSCs-CM combined with zinc sulfate, we observed weight restoration and normalization of blood glucose levels in both treated groups compared to the diabetic control groups. Previous diabetes-related studies have highlighted the importance and impact of stem cell therapies, including MSCs and ADSCs, in regulating body weight and blood glucose levels (Takafuji et al., 2021).

In our study, mice treated with ADSC-CM, along with those treated with ADSC-CM combined with zinc sulfate, demonstrated improved wound closure compared to the diabetic control group. Conversely, healthy mice exhibited a normal healing process. Previous studies have highlighted the significance of ADSC-CM, containing essential elements crucial for wound healing, particularly in enhancing the healing process of chronic diabetic wounds (Guo et al., 2022).

MMPs play a significant role in extracellular matrix (ECM) remodeling and are crucial factors in wound healing. These enzymes can degrade various ECM components and facilitate cell migration. Therefore, a regulated activation and inhibition cascade of *MMPs* are necessary for efficient wound closure (Dai et al., 2021). Our study revealed higher expression of *MMP-2* in diabetic control wounds compared to both ADSC-CM treated wounds and healthy control wounds. This suggests a greater breakdown of ECM in diabetic wounds, potentially contributing to delayed healing. Similar findings from previous studies have indicated elevated *MMPs* expression in diabetic wounds and ulcers (Amadio et al., 2021). Our results suggest that ADSC-CM treatment may help reduce the increased expression of *MMPs* in diabetic wounds, presenting a novel approach in the treatment strategies for diabetic ulcers.

Previous studies have suggested that adipose stem cells (ASCs) initiate skin wound repair through differentiation into skin cells (Strong et al., 2015). The self-renewal and differentiation of stem cells, crucial for wound healing, are regulated by various transcription factors (TFs), including *NANOG* and *OCT4* (Strong et al., 2015). Our study revealed increased expression levels of both *NANOG* and *OCT4*in the ADSC-CM treatment group compared to the diabetic control group. This suggests that ADSC-CM may enhance the stemness and differentiation capabilities of local stem cells, thereby promoting the wound repair process. Previous

research has highlighted the roles of both *NANOG* and *OCT4* in wound repair and tissue remodeling (Wollenzien et al., 2018). The increased expression of these TFs in ASCs indicates efficient therapeutic potential (Dentelli et al., 2013).

Wound healing, immune response, and inflammation are intricately connected. Zinc has been noted to modulate both innate and adaptive immune responses, and its deficiency is linked to delayed wound healing (Lin et al., 2017). Additionally, zinc sulfate plays a crucial role in mitosis, cell proliferation, and DNA synthesis (Mann et al., 2016), making it a vital element for proper wound healing. Our study combined zinc sulfate with ADSC-CM and observed that the combined dose (Zinc sulfate+ADSC-CM) was more effective in promoting wound closure and reducing blood glucose levels compared to the ADSC-CM (pure) group. These results emphasize the significance of utilizing ADSC-CM either as a single dose or synergistically with zinc sulfate for treating diabetic wounds.

CONCLUSION

ADSCs-CM has emerged as an attractive treatment for diabetes-associated wounds due to its potential in accelerating the wound healing process. Our study demonstrated that applying ADSCs-CM around the wound bed in diabetic mice resulted in enhanced wound healing and a reduction in blood glucose levels compared to the control groups. Furthermore, the application of ADSCs-CM regulated the expression of various crucial genes, including *MMP-2*, *NANOG*, and *OCT4*, known to be associated with the wound healing process. In conclusion, our study confirms the effectiveness of ADSCs-CM therapy and supports its consideration as a viable treatment option for diabetic wounds.

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CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interests.

REFERENCES

1. Alrouji M, Kuriri FA, Alqasmi MH, AlSudais H, Alissa M, Alsuwat MA, Asad M, Joseph B, Almuhanna Y (2023). A Simple In-Vivo Method for Evaluation of Antibiofilm and Wound Healing Activity Using Excision Wound Model in Diabetic Swiss Albino Mice. Microorg. 11:692.
2. Amadio EM, Marcos RL, Serra AJ, Dos Santos SA, Caires JR,

Fernandes GHC, Leal-Junior EC, Ferrari JCC, de Tarso Camillo de Carvalho P (2021). Effect of photobiomodulation therapy on the proliferation phase and wound healing in rats fed with an experimental hypoproteic diet. Lasers Med. Sci. 36:1427-1435.

3. Athanassiou P, Athanassiou L, Kostoglou-Athanassiou I (2020). Nutritional pearls: diet and rheumatoid arthritis. Mediterr. J. Rheumatol. 31:319.
4. Brem H, Sheehan P, Rosenberg HJ, Schneider JS, Boulton AJ (2006). Evidence-based protocol for diabetic foot ulcers. Plast. Reconstr. Surg. 117:193S-209S.
5. Chen RF, Wang CT, Chen YH, Chien CM, Lin SD, Lai CS, Wang CJ, Kuo YR (2019). Hyaluronic acid–povidone-iodine compound facilitates diabetic wound healing in a streptozotocin-induced diabetes rodent model. Plast. Reconstr. Surg. 143(1): 1371-1382.
6. Chen T, Qin Y, Chen M, Zhang Y, Wang X, Dong T, Chen G, Sun X, Lu T, White RA (2021). Gestational diabetes mellitus is associated with the neonatal gut microbiota and metabolome. BMC Med. 19(1): 1-10.
7. Cheng YW, Nicholson JM, Nakagawa S, Bruckner TA, Washington AE, Caughey AB (2008). Perinatal outcomes in low-risk term pregnancies: do they differ by week of gestation? Am. J. Obstet. Gynecol. 199: 370. e371-370. e377.
8. Dai J, Shen J, Chai Y, Chen H (2021). IL-1β impaired diabetic wound healing by regulating MMP-2 and MMP-9 through the p38 pathway. Mediators Inflamm. 2021: 1-10.
9. Davis FM, Kimball A, Boniakowski A, Gallagher K (2018). Dysfunctional wound healing in diabetic foot ulcers: new crossroads. Curr. Diab. Rep. 18:1-8.
10. Dentelli P, Barale C, Togliatto G, Trombetta A, Olgasi C, Gili M, Riganti C, Toppino M, Brizzi MF (2013). A diabetic milieu promotes OCT4 and NANOG production in human visceral-derived adipose stem cells. Diabetologia. 56(1): 173-184.
11. Dewangan H, Tiwari RK, Sharma V, Shukla SS, Satapathy T, Pandey R (2017). Past and Future of in-vitro and in-vivo Animal Models for Diabetes: A Review. Ind. J. Pharm. Educ. Res. 51: s522-s530.
12. Dewey S (2014). Fluctuating Effects of Diabetes Mellitus Type 1 and 2 on the Ubiquitin Proteasome System in Two Rodent Models: Proteasome Inhibition is Not the Magic Answer. University of California, Davis,

13. DiMarino AM, Caplan AI, Bonfield TL (2013). Mesenchymal stem cells in tissue repair Front. Immunol. 4(1): 201.
14. Fowler MJ (2008). Microvascular and macrovascular complications of diabetes. Clin. Diabet. 26 (1):77-82.
15. Game F, Hinchliffe R, Apelqvist J, Armstrong D, Bakker K, Hartemann A, Löndahl M, Price PE, Jeffcoate W (2012). A systematic review of interventions to enhance the healing of chronic ulcers of the foot in diabetes. Diabet. Metab. Res. Rev. 28(1): 119-141.
16. Guo X, Schaudinn C, Blume-Peytavi U, Vogt A, Rancan F (2022). Effects of adipose-derived stem cells and their conditioned medium in a human ex vivo wound model. Cells. 11: 1198.
17. Hernandez L, Leutwyler H, Cataldo J, Kanaya A, Swislocki A, Chesla C (2019). The Symptom Experience of Older Adults with Type 2 Diabetes and Diabetes-related Distress.Nurs. Res. 68: 374.
18. Kharroubi AT, Darwish HM (2015). Diabetes mellitus: The epidemic of the century. World J. Diabet. 6:850.
19. Lewis GF, Brubaker PL (2021). The discovery of insulin revisited: lessons for the modern era. J. Clin. Investig. 131.
20. Lin PH, Sermersheim M, Li H, Lee PH, Steinberg SM, Ma J (2017). Zinc in wound healing modulation. Nut. 10:16.
21. Lin SY, Huang HA, Lin SC, Huang YT, Wang KY, Shi HY (2016). The effect of an anaesthetic patient information video on perioperative anxiety: A randomised study. Eur. J. Anaesthesiol. 33:134-139.
22. Lupu-Haber Y, Bronshtein T, Shalom-Luxenburg H, D'Atri D, Oieni J, Kaneti L, Shagan A, Hamias S, Amram L, Kaneti G (2019). Pretreating mesenchymal stem cells with cancer conditioned-media or proinflammatory cytokines changes the tumor and immune targeting by nanoghosts derived from these cells. Adv. Healthc. Mater. 8(1): 1801589.
23. Lux CN (2022). Wound healing in animals: a review of physiology and clinical evaluation. Vet. Dermatol. 33(1): 91-e27.
24. Mann TH, Seth Childers W, Blair JA, Eckart MR, Shapiro L (2016). A cell cycle kinase with tandem sensory PAS domains integrates cell fate cues. Nat. Commun. 7(1): 11454.
25. Mazini L, Rochette L, Admou B, Amal S, Malka G (2020). Hopes and limits of adipose-derived stem cells (ADSCs) and mesenchymal stem cells (MSCs) in wound healing. Int. J. Mol. Sci. 21: 1306.

26. Menke MN, Menke NB, Boardman CH, Diegelmann RF (2008). Biologic therapeutics and molecular profiling to optimize wound healing. J. Gynecol. Oncol. 111(1): S87-S91.
27. Ogurtsova K, da Rocha Fernandes J, Huang Y, Linnenkamp U, Guariguata L, Cho NH, Cavan D, Shaw J, Makaroff L (2017). IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabet. Res. Clin. Pract. 128(1): 40-50.
28. Olisah CC, Smith L, Smith M (2022). Diabetes mellitus prediction and diagnosis from a data preprocessing and machine learning perspective. Comput. Methods Programs Biomed. 220:106773.
29. Spampinato SF, Caruso GI, De Pasquale R, Sortinom MA, Merlo S (2020). The treatment of impaired wound healing in diabetes: looking among old drugs. Pharm J. 13:60.
30. Standl E, Khunti K, Hansen TB, Schnell O (2019). The global epidemics of diabetes in the 21st century: Current situation and perspectives. Eur. J. Prev. Cardiol. 26(1): 7-14.
31. Strong AL, Bowles AC, MacCrimmon CP, Frazier TP, Lee SJ, Wu X, Katz AJ, Gawronska-Kozak B, Bunnell BA, Gimble JM (2015). Adipose stromal cells repair pressure ulcers in both young and elderly mice: potential role of adipogenesis in skin repair. Stem Cells Transl. Med. 4: 632-642.
32. Szunerits S, Melinte S, Barras A, Pagneux Q, Voronova A, Abderrahmani A, Boukherroub R (2021). The impact of chemical engineering and technological advances on managing diabetes: Present and future concepts. Chem. Soc. Rev. 50:2102-2146.
33. Takafuji Y, Tatsumi K, Kawao N, Okada K, Muratani M, Kaji H (2021). MicroRNA-196a-5p in extracellular vesicles secreted from myoblasts suppresses osteoclast-like cell formation in mouse cells. Calcif. Tissue Int. 108(1): 364-376.
34. Than UTT, Guanzon D, Wager L, Manton KJ, Hollier B, Leavesley D (2015). An analysis of exosomes from keratinocytes and fibroblasts. Biomed. Eng. Online. Springer. 137-141.
35. Wollenzien H, Voigt E, Kareta MS (2018). Somatic pluripotent genes in tissue repair, developmental disease, and cancer. SPG biomed. 1.
36. Zheng S, Fan J, Yu F, Feng B, Lou B, Zou Q, Xie G, Lin S, Wang R, Yang X (2020). Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. Bio. Med. J. 369