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Research Article
Secretome of Human MSC Gel Improves DFU Healing through NF-κB p50 and CD163 mRNA Expression

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Abstract

Background and Objective: Diabetic foot ulcers (DFUs) remain a critical clinical problem and stem cell-derived secretome reared under hypoxic conditions has been shown to play a significant role in tissue repair via immunomodulation. This study aimed to evaluate the secretome of human mesenchymal stem cell gel (SH-MSC gel) in DFU patients with grades 2 and 3 through reduced wound volume and modulation of CD163 and NF-κB p50 mRNA expression. **Materials and Methods:** A prospective, randomized controlled clinical trial involved 16 DFU patients with grades 2 and 3. Participants received either a placebo gel or an intervention gel containing secretome from Human Umbilical Cord Mesenchymal Stem Cells (hUC-MSCs) cultured under hypoxic conditions. All patients received standard wound care. Primary outcomes included changes in wound volume and expression levels of CD163 and NF-κB p50 mRNA in wound tissue, assessed using quantitative PCR. The Shapiro-Wilk test assessed normality and for normally distributed data, paired t-tests (within-group) and unpaired t-tests (between-group) were used. One-way ANOVA compared means across groups, while the Kruskal-Wallis test followed by *post hoc* analysis was employed for non-parametric data ($p<0.05$). Statistical analysis was performed using GraphPad Prism 10. **Results:** Baseline characteristics of participants did not show significant differences between the groups. Treatment with SH-MSC gel significantly enhanced wound healing compared to the placebo group, evidenced by a marked reduction in wound volume after 7 days (95% CI (0.467 to 1.18), $p<0.001$). The CD163 mRNA expression significantly increased in the SH-MSC gel group post-treatment (95% CI (-2.20 to -1.11), $p<0.001$), while NF-κB p50 mRNA expression significantly decreased (95% CI (0.349 to 0.688), $p<0.001$). **Conclusion:** The clinical trial results suggested that SH-MSC gel effectively improves wound healing in DFUs. Further research is warranted to explore additional inflammatory markers to better understand DFU treatment.

Key words: Diabetic foot ulcers, stem cells, wound healing, NF-κB p50, CD163 antigen

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetic foot ulcers (DFUs) represent a significant clinical challenge, with high morbidity and substantial economic impact globally[1,2]. Recent data show a two-year cumulative incidence of 8% and an incidence rate of 49.9 per 1000 person-years. The one-year incidence rate is 1.8%, which increases to 4.18% in the second year[3]. These chronic wounds are often caused by peripheral neuropathy and impaired angiogenesis, common complications in type 2 diabetes mellitus[4]. Current treatment options for DFUs, including standard wound care, offloading and topical agents, are often ineffective. Standard wound care achieves only 20-30% healing rates within 12 weeks[5]. Topical agents like silver sulfadiazine and hydrocolloid dressings have shown inconsistent results, sometimes demonstrating no significant improvement compared to placebo treatments[6]. These limitations highlight the need for novel therapeutic strategies to enhance wound healing.

Recent advances in regenerative medicine suggest that while mesenchymal stem cells (MSCs) have the potential to improve DFU healing through tissue repair and inflammation modulation, the use of MSC-derived secretome, especially when cultured under hypoxic conditions, presents an innovative alternative. Rather than relying on the cells, the secretome has demonstrated enhanced efficacy in promoting angiogenesis and modulating inflammatory responses, potentially offering a more efficient and targeted approach to wound healing[7,8]. The NF-κB signaling pathway, especially the p50 subunit and CD163 mRNA expression play key roles in inflammation resolution and tissue remodeling[9,10]. Despite their potential, MSC treatments face challenges such as variable efficacy and difficulties in translating preclinical successes to clinical outcomes[11]. This study aimed to overcome these issues by optimizing MSC delivery methods and evaluating their effects on specific macrophage subtypes involved in wound healing. This study aims to investigate the role of NF-κB p50 and CD163 mRNA expression in the reduction of wound volume induced by the Secretome of Human Mesenchymal Stem Cell Gel (SH-MSC gel) in diabetic foot ulcers.

MATERIALS AND METHODS

Study design: This randomized, prospective, controlled clinical trial included two groups: A control group treated with a placebo Base gel and an intervention group treated with SH-MSC gel, produced by the Stem Cell and Cancer Research Center, Indonesia. The study was carried out from November, 2023 to May, 2024.

The MSCs were isolated from Human Umbilical Cord-Derived MSCs (hUC-MSCs) and obtained with informed consent from donors post-childbirth. The umbilical cords were processed to isolate MSCs, which were then cultured under hypoxic conditions (1-5% oxygen) to enhance their therapeutic potential.

Characterization of the MSCs was performed using flow cytometry to confirm the presence of markers like CD73, CD90 and CD105 and the absence of hematopoietic markers such as CD34 and CD45. The MSC's differentiation potential was also assessed. For secretome production, MSCs were cultured to confluence, then incubated in serum-free medium under hypoxia for 24-48 hrs to produce a secretome rich in bioactive molecules.

The trial was conducted at Sultan Agung Islamic Hospital, Semarang, Indonesia. The SH-MSC gel, containing 200 µL of secretome at a 20% concentration, was applied topically to wounds daily before sleep for 2 weeks. The dosage was based on preclinical studies showing optimal wound healing at this concentration. The nighttime application was chosen to reduce gel displacement and take advantage of the body's natural repair processes during sleep[12].

Participants: Sixteen participants with type 2 diabetes mellitus (DM) and grade 2-3 diabetic foot ulcers (DFUs) were included based on Table 1, which summarizes the inclusion and exclusion criteria used to select participants for the study.

Demographic and clinical characteristics: The study initially included 20 participants with type 2 diabetes mellitus (DM) who had grade 2-3 diabetic foot ulcers, as classified by the Wagner scale. However, 4 subjects were excluded before the study began due to ineligibility based on the criteria. Ultimately, 16 participants were included in the study, with 10 having grade 2 DFUs and 6 having grade 3 DFUs (Fig. 1). The participants were evenly divided into two groups: 8 received the SH-MSC gel treatment and 8 received the Base gel placebo.

Preparation, flow cytometry phenotyping and differentiation analysis of UC-MSCs: Isolation of UC-MSCs was carried out as previously mentioned by Sanjeeviraj *et al.*[1]. The cells were grown at 37°C and 5% CO_2 in DMEM low glucose (Gibco, New York, USA) supplemented with 10% FBS, 1.5% penicillin/streptomycin and 0.25% amphotericin B (Gibco). The culture medium was changed every three days. For later research, UC-MSCs from the fifth passage were used. Human anti-CD90-FITC, CD105-perCP, CD73-APC and Lin-PE antibodies were used in flow cytometry analysis to describe the surface markers on UC-MSCs at the fifth passage

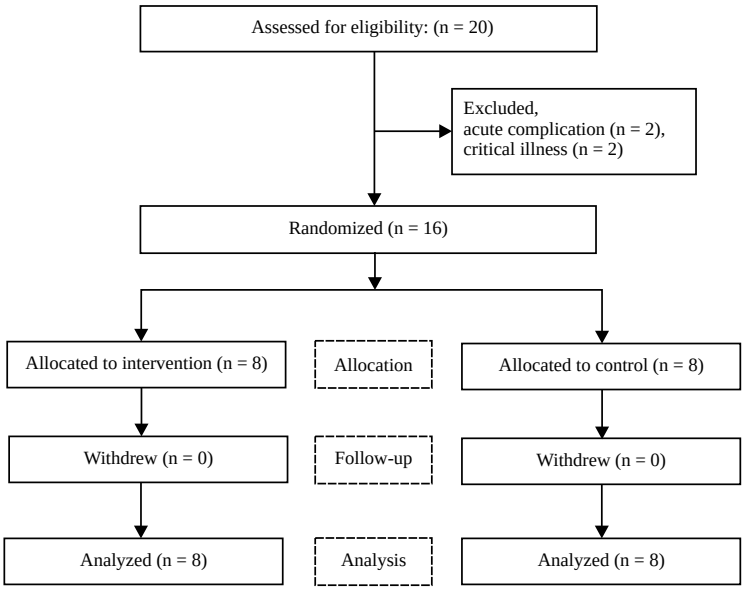

Fig. 1: Enrolment and outcome of participants in the clinical trial

Table 1: Inclusion and exclusion criteria of the participants

Inclusion criteria	Exclusion criteria
Patients over 40 years old	Liver cirrhosis
Albumin level above 3 mg/dL	Critical illnesses (acute stroke, acute myocardial infarction)
HbA1c level over 8%	Critical limb ischemia
Hemoglobin level above 10% (mg)	Acute complications (diabetic ketoacidosis, hyperosmolar hyperglycaemic state, hypoglycaemia)
	Pregnancy or breastfeeding

(BD Bioscience, California, USA). A BD FACS Lyric flow cytometer and associated software (BD Bioscience) were used to observe and analyse the cells. Using a standard medium at 37°C and 5% CO^2, the adipogenic and chondrogenic differentiation of UC-MSCs in the fifth passage was also investigated. Both the osteogenic and adipogenic MesenCult™ baseline medium (Stem Cell Technologies, Singapore) and each supplement (Stem Cell Technologies) were added, along with 0.25% amphotericin B (Gibco), 1% penicillin (Gibco) and 1% L-glutamine (Stem Cell Technologies). After 21 days, lipid and calcium deposits were stained using oil red O and alizarin red staining (Sigma-Aldrich, Missouri, USA). The media was replaced every three days.

Hypoxia preconditioning in MSCs: After achieving 80% confluence, UC-MSCs were put in a hypoxic chamber (Stem Cell Technologies) and maintained at a 5% oxygen concentration using an oxygen controller (BioSpherix, Lacona, New York, USA) that measured the Partial Pressure of Oxygen (PO_2). The cells were maintained in the compartment with 5% CO_2 and 37°C for a whole day. The culture media was gathered once the incubation was complete.

SH-MSCs preparation and profiling: After centrifuging the MSCs under hypoxic circumstances for 10 min at 4°C at 13,000 g, the culture media was removed from the CM of the cells. As previously mentioned in our work, the tangential flow filtration (TFF) method was applied to isolate SH-MSCs[2]. Filter cassettes having hole diameters of 10-30 kDa (50%), 30-50 kDa (25%) and 300-500 kDA (25%), were used to filter the H-MSC-CM molecules. Enzyme-Linked Immunosorbent Assay (ELISA) kits for IL-10, TGF-β and VEGF (Invitrogen, California, USA) were used to evaluate the cytokine and growth factor levels in SH-MSCs by the manufacturer's instructions. A microplate reader (Bio-Rad, California, USA) with a 450 wavelength was used to evaluate the data.

CD163 and p50 gene expression analysis: As directed by Sigma Aldrich, 50 mg of tissue samples that were taken on day 9 were processed to extract total RNA using TRI reagent. The Enhanced Avian First Strand cDNA Synthesis kit was used and the cDNA synthesis was carried out according to the manufacturer's instructions (Sigma-Aldrich). Using an oligo d(T) primer, reverse transcription was carried out and the sample was incubated for 10 min at 70°C and then for 15 min at 45°C. Using the KAPA SYBR® FAST Universal

Kit (Sigma Aldrich), 3 ng of cDNA template and primers (GAPDH: Forward 5'-ACTCCACTCACGGCAAATTC-3' and reverse 5'-TCTCCATGGTGGTGAAGACA-3'; CD163: Forward 5'-GGATCTGCTGACTTCAGAAG-3' and reverse 5'-CTCCTTGT CTGTTCCTCCAA-3'; p50: Forward 5'-GCAGATGGCCCATACC TTCA-3' and reverse 5'-CACCATGTCCTTGGGTCCAG-3'), a 2-step quantitative real-time PCR was carried out using an Eco Real-Time PCR System (Illumina Inc., San Diego California, USA) to assess the expression levels of CD163 and p50. The temperature for thermocycling was set to 95°C for 3 min, then 40 cycles of 95°C for 10 sec and 60°C for 30 sec. The ΔΔCt technique was utilized to quantify the expression of CD163 and p50 genes using the Eco Study Software (Illumina).

Intervention: The intervention involved SH-MSC gel, with confirmed positive markers $CD73^+$, $CD90^+$ and $CD105^+$ and negative markers $CD45^-$, $CD34^-$, $CD11b^-$ and HLA-DR^-. The placebo was Base gel. Inflammatory and growth parameters in this study were assessed using 50 mg tissue samples. The analysis was employed using ELISA assay. Both gels are produced by Stem Cell and Cancer Research, Semarang, Indonesia.

Study procedure: The study initially included 20 participants with type 2 diabetes mellitus (DM) who had grade 2-3 diabetic foot ulcers, as classified by the Wagner scale. Four subjects were excluded before the study began due to ineligibility based on the criteria. Ultimately, 16 participants were included in the study, with 10 having grade 2 DFUs and 6 having grade 3 DFUs. The participants were evenly divided into two groups: 8 received the SH-MSC gel treatment and 8 received the Base gel placebo. Participants were randomized into two groups. Pre-treatment assessments included wound volume and mRNA expression of NF-κB p50 and CD163. The control group received standard therapy, while the intervention group received 30 mL of SH-MSC gel. The CD163 and NF-κB p50 expression were measured after 7 days and wound healing parameters were evaluated after 7 and 14 days.

Statistical analysis: Descriptive statistics summarize categorical and numerical data. The normality of the data was assessed using the Shapiro-Wilk test. Data of this study is normally distributed and summarized as Mean±SD. For within-group comparisons and paired t-tests this study used normally distributed variables (parametric). For this study, group comparisons and unpaired t-tests were used for parametric data. One-way ANOVA was employed to compare means across groups for parametric variables, while the Kruskal-Wallis test was used for non-parametric variables, followed this study by *post hoc* analysis where significant results this study found ($p<0.05$). Statistical analysis was performed using GraphPad Prism 10.

Ethical consideration: This study was approved by the Ethical Committee of Sultan Agung Islamic Hospital, Semarang, Indonesia (No. 2/KEPK-RSISA/I/2024). Informed consent was obtained from all participants before their enrollment. All data this handled per the principles of confidentiality and participant rights.

RESULTS

MSCs characteristics: In compliance with ISCT (International Society for Cellular Therapy) recommendations, this study assessed the cell shape, expression of membrane markers and differentiation potential of UC-MSCs at the fifth passage to identify their properties. The cells had a spindle-shaped characteristic and resembled fibroblasts (Fig. 2a). The ability of UC-MSCs to differentiate was verified by adipogenic (Fig. 2b) and osteogenic (Fig. 2c) differentiation tests. The UC-MSCs in this study, 21 days of incubation before differentiating into osteocytes and adipocytes, as evidenced by the red deposits of calcium and lipid, respectively. The cells' immunophenotyping profile studied that CD90, CD73 and CD105 and Lin showed positive expression (Fig. 2d).

Molecules contained in SH-MSCs: After MSCs, this study was cultured for 24 hrs in a hypoxic setting and the CM from H-MSCs was extracted. To obtain purified SH-MSCs, using a TFF technique based on molecular study might cut-off categories to separate the molecules contained in the H-MSC-CM, as previously reported. To separate the molecules, this study used filter cassettes of 10-30 kDa (50%), 30-50 kDa (25%) and 300-500 kDA (25%) sizes. Following filtration, this study measured the amount of anti-inflammatory cytokines (Table 2) in SH-MSCs using ELISA. The concentrations of TGF-β, VEGF and IL-10 in this study showed 558.73±10.32, 242.58±7.55 and 1151.00±21.20 pg/mL, in that order.

Demographic and clinical characteristics: Various clinical parameters detailed the characteristics of the participants. All patients in each group had similar baseline characteristics, including age, HbA1c levels and other relevant metrics (Table 3). Before conducting statistical analysis, the data was tested for normality using the Shapiro-Wilk test and for

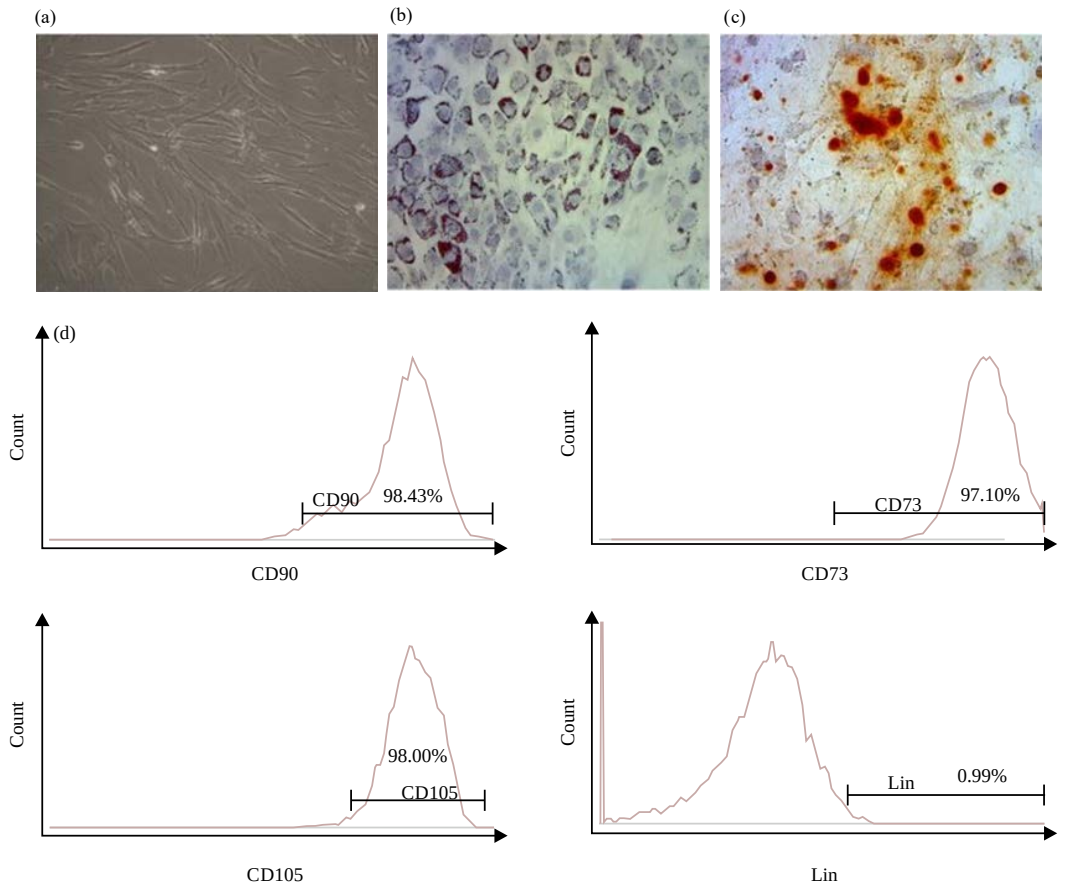

Fig. 2(a-d): Properties of MSCs, (a) Resembled fibroblasts, (b) Adipogenic differentiation test, (c) Osteogenic differentiation test and (d) CD90, CD73, CD105 and Lin positive expression

Clear overview of the participant's journey through the clinical trial, highlighting the screening, randomization and outcome assessment

Table 2: Analysis of anti-inflammatory molecule contained in SH-MSCs

Molecules	SH-MSCs value±SE (pg/mL)
IL-10	558.73±10.32
TGF-β	242.58±7.55
VEGF	1151.00±21.20

Analysis was employed using an ELISA assay

homogeneity of variances using Levene's test. Variables that passed both tests of this study, were analyzed using parametric methods, while those that did not meet the assumptions of normality or homogeneity were analyzed using non-parametric methods.

The study compared the clinical features of studying placebo and SH-MSC gel groups, showing no statistically significant differences in key parameters, including age, hemoglobin, leucocyte count, albumin, HbA1c and random blood glucose levels. Gender distribution varied groups, with a higher proportion of females in the SH-MSC gel group. The findings suggest comparable baseline characteristics across the groups ($p>0.05$).

Efficacy profile: The study evaluated the effects of SH-MSC gel on wound volume, CD163 mRNA expression and NF-κB p50 mRNA expression in diabetic foot ulcers (Fig. 3a-c). On day 0, no significant difference in wound volume was observed and the base gel and SH-MSC gel groups (95% CI -0.283 to 0.433, $p = 0.675$; Fig. 3d). The SH-MSC gel group showed a significant reduction in wound volume by day 7 (95% CI 0.467 to 1.18, $p<0.001$) and day 14 (95% CI 0.792 to 1.20, $p<0.001$; Fig 3e), compared to day 0. In contrast, the base gel group showed no significant changes in wound volume across all time points ($p>0.05$; Fig 3d). This result summarizes the differences in wound volume and gene expression of treatment groups and across time points (Table 4). The secretome gel group showed the mean wound volume and NF-κB p50 mRNA expression but higher CD163 mRNA expression compared to the base gel group. Variability, as indicated by the standard deviation, was higher in the secretome gel group across all parameters (Table 5).

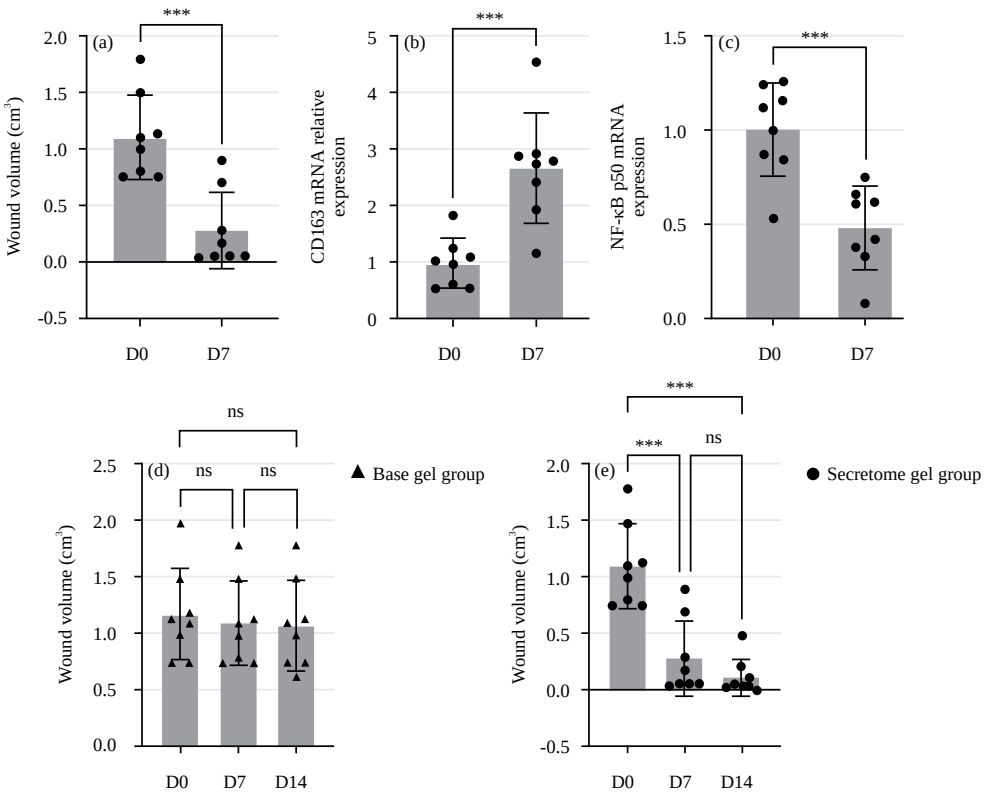

Fig. 3(a-e): Efficacy of treatment of wound healing and inflammatory markers, (a) Wound volume measurement on (D0) and (D7), (b) Relative expression of CD163 mRNA on D0 and D7, (c) NF-κB p50 mRNA expression levels on D0 and D7, (d) Wound volume changes over time in the base gel group (D0, D7 and D14) and (e) Wound volume reduction in the secretome gel group

(a) Significant reduction after treatment (***p<0.001), (b) Reduced inflammation (***p<0.001), (c) Significant downregulation (***p<0.001), (d) No significant differences, (e) Significant healing on D7 and D14 compared to D0 (***p<0.001, ns: Not significant than D7 and D14). ns: Not significant (p>0.05) and ***Significant (p≤0.05)

Table 3: Baseline demographic and clinical features of participants

Clinical features	Placebo gel group	SH-MSC gel group	p-value
N	8	8	-
Gender*			
Male, n (%)	6 (75)	3 (37.5)	-
Female, n (%)	2 (25)	5 (62.5)	-
Age (year)	61.13±3.80	58.63±3.21	0.62
Hemoglobin (g/dL)	11.5±0.34	12.1±0.52	0.32
Leucocyte (10^3/μL)	9.64±0.40	9.20±0.52	0.52
Albumin (g/dL)	4.12±0.26	3.92±0.14	0.52
HbA1c (%)	7.84±0.69	8.38±0.45	0.53
Random blood glucose (g/dL)	193±28	177±19	0.32

Continuous data are presented as Mean±SD, p-values this study calculated using unpaired t-tests for continuous variables, no statistically significant differences in this study were found in the Placebo gel group and SH-MSC gel group for any of the clinical characteristics (all p>0.05), The data of this study is normally distributed as confirmed by the Shapiro-Wilk test, which justified the use of unpaired t-tests and *p-value (Chi-square test with Yates' correction): 0.31

For inflammatory markers, wound volume measurement on day 0 (D0) and day 7 (D7), showed significant reduction after treatment (p<0.001) Fig. 3a. The SH-MSC gel group exhibited a significant increase in CD163 mRNA expression by day 7 (95% CI (-2.20 to -1.11), p<0.001; Fig 3b), indicative of reduced inflammation. Furthermore, NF-κB p50 mRNA expression significantly decreased by day 7 in the SH-MSC gel group (95% CI (0.349 to 0.688), p<0.001; Fig 3c), suggesting an anti-inflammatory effect. No significant changes this observed in CD163 or NF-κB p50 mRNA expression within the base gel group at any time point (p>0.05; Fig 3b-c). Wound volume changes over time in the base gel group (D0, D7 and D14),

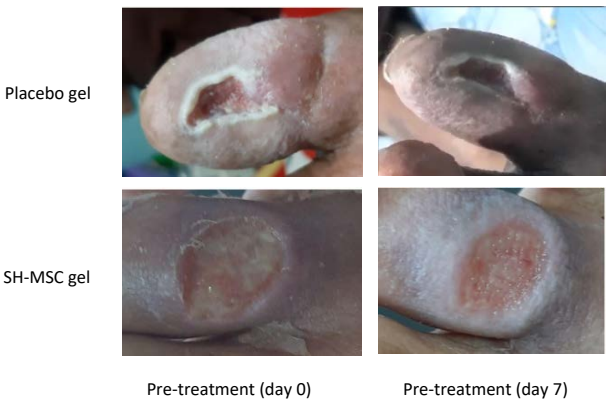

Fig. 4: Wound of DFUs patients before and after intervention

Table 4: Effects of base gel and secretome gel on wound healing and gene expression over time

Parameter	Comparison	Mean difference	95% CI	Significant	p-value	Summary
Wound volume						
Day 0	Base gel vs secretome gel	0.075	-0.283 to 0.433	No	0.675	ns
Day 7	Base gel vs secretome gel	0.826	0.467 to 1.18	Yes	<0.001	***
Day 14	Base gel vs secretome gel	0.974	0.616 to 1.33	Yes	<0.001	***
Base gel	Day 0 vs day 7	0.075	-0.129 to 0.279	No	0.457	ns
	Day 0 vs day 14	0.097	-0.107 to 0.301	No	0.339	ns
	Day 7 vs day 14	0.022	-0.182 to 0.226	No	0.828	ns
Secretome gel	Day 0 vs day 7	0.826	0.622 to 1.03	Yes	<0.001	***
	Day 0 vs day 14	0.996	0.792 to 1.20	Yes	<0.001	***
	Day 7 vs day 14	0.171	-0.033 to 0.374	No	0.098	ns
CD163 mRNA expression						
Day 0	Base gel vs secretome gel	0.034	-0.510 to 0.577	No	0.900	ns
Day 7	Base gel vs secretome gel	-1.66	-2.20 to -1.11	Yes	<0.001	***
Base gel	Day 0 vs day 7	0.000	-0.575 to 0.575	No	>0.999	ns
Secretome gel	Day 0 vs day 7	-1.69	-2.26 to -1.12	Yes	<0.001	***
NF-κB p50 mRNA expression						
Day 0	Base gel vs secretome gel	-0.003	-0.172 to 0.167	No	0.976	ns
Day 7	Base gel vs secretome gel	0.519	0.349 to 0.688	Yes	<0.001	***
Base gel	Day 0 vs day 7	0.000	-0.154 to 0.154	No	>0.999	ns
Secretome gel	Day 0 vs day 7	0.521	0.368 to 0.675	Yes	<0.001	***

ns: Not significant, *Significant at p<0.05, ****Significant at p<0.01 and ***Significant at p<0.001

with no significant differences Fig. 3d. Wound volume reduction in the secretome gel group, showing significant healing on D7 and D14 compared to D0 Fig. 3e. Wound of DFUs patients before and after intervention can be shown in Fig. 4 in pre-treatment of day 0 and post-treatment of day 7 treated with placebo gel and SH-MSC gel, respectively.

The scatterplots (Fig. 5a-c) demonstrate the relationships of this study wound volume changes, CD163 and NF-κB p50 expression in diabetic foot ulcer patients. Figure 5a shows a positive correlation between wound volume change and CD163 expression ($r = 0.17$, $p = 0.52$), indicating no significant relationship of these variables. Figure 5b similarly shows the correlation of wound volume change and NF-κB p50 expression ($r = 0.02$, $p = 0.95$), suggesting that NF-κB p50 expression does not significantly impact wound size. In contrast, Fig. 5c reveals a strong negative correlation between this study CD163 and NF-κB p50 expression changes ($r = -0.62$, $p = 0.01$), suggesting that higher CD163 expression is linked to reduced NF-κB p50 levels, potentially modulating inflammation in diabetic foot ulcers.

DISCUSSION

Mesenchymal stem cells (MSCs) have emerged as a promising therapy for skin wounds and ulcers due to their potent regenerative capabilities. In addition, the secretome of MSCs, which contains a rich array of molecular factors, has demonstrated significant potential in promoting tissue regeneration and repair. Based on this evidence, the present work evaluated the effects of SH-MSC gel on wound healing in diabetic foot ulcers. The SH-MSC gel may hold great therapeutic applications in the healing of diabetic foot ulcers.

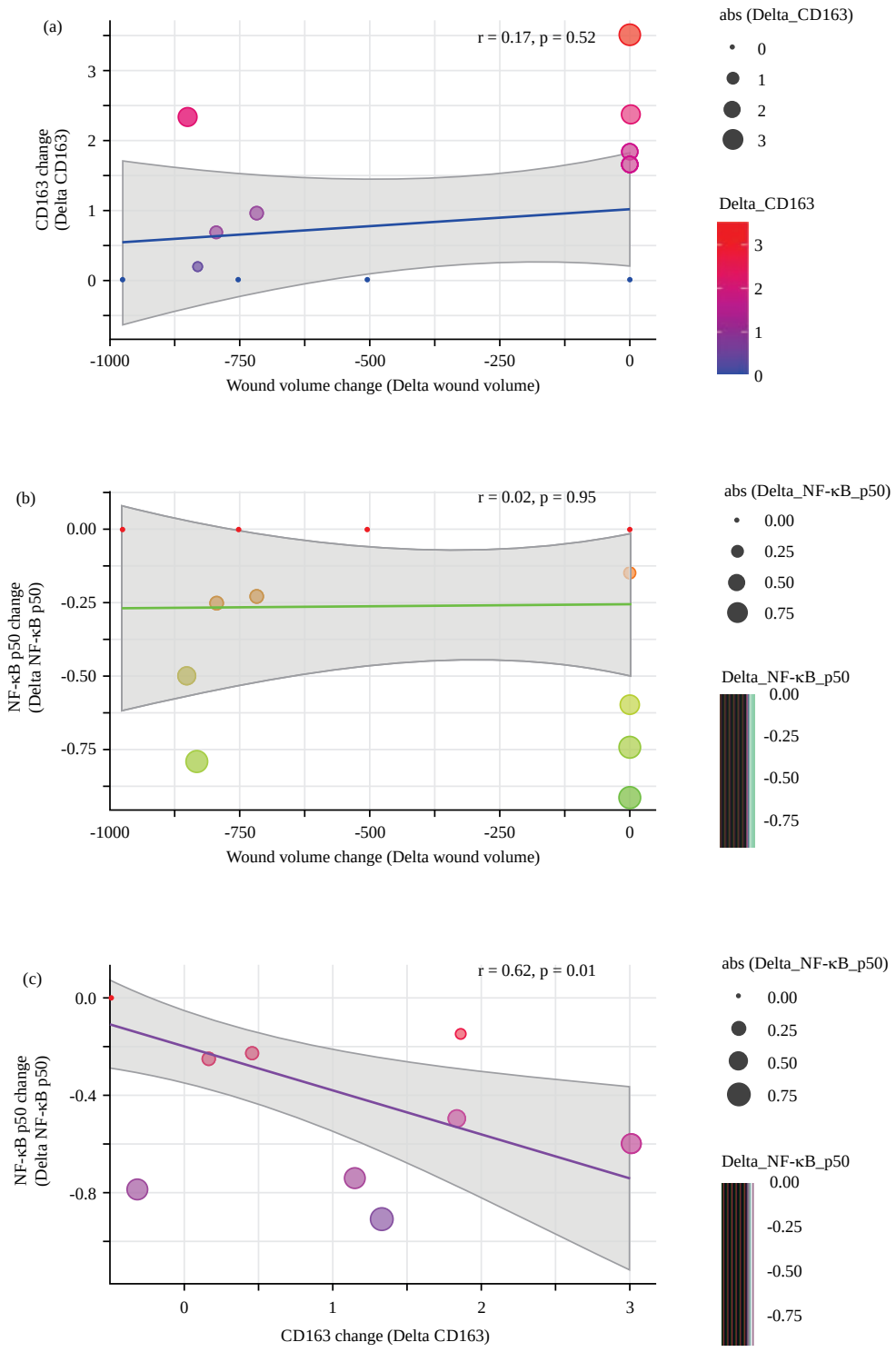

Fig. 5(a-c): Correlations wound volume changes in CD163 and NF-κB p50 mRNA expression in DFU patients, (a) Correlation of this study wound volume change and CD163 mRNA expression change (r = 0.17, p = 0.52), (b) Correlation in this study wound volume change and NF-κB p50 mRNA expression change (r = 0.02, p = 0.95) and (c) Correlation of this study CD163 mRNA and NF-κB p50 mRNA expression changes (r = -0.62, p = 0.01)
Points are color-coded to represent delta values for each variable, with size indicating absolute change

Table 5: Comparison of wound volume and gene expression in this study of Base gel and secretome gel groups

Parameter	Statistic	Base gel (n = 8)	Secretome gel (n = 8)
Wound volume	Mean	1.14	0.693
	Standard deviation	0.383	0.549
	Standard error mean	0.0958	0.137
NF-κB p50 mRNA expression			
CD163 mRNA expression	Mean	1.00	0.742
	Standard deviation	0.00	0.351
	Standard error mean	0.00	0.0879
	Mean	1.00	1.81
	Standard deviation	0.00	1.13
	Standard error mean	0.00	0.284

The comparative changes in wound size, NF-κB p50 and CD163 mRNA expression provide a holistic view of how SH-MSC gel stimulates the wound healing process at the molecular level[13,14].

The present study demonstrates the promising therapeutic potential of SH-MSC gel in the treatment of diabetic foot ulcers (DFUs). The observed decrease in wound volume in the SH-MSC gel group suggests its effectiveness in enhancing wound healing, particularly in DFUs, which are known for their treatment challenges and complications[15]. The therapeutic effects of secretome gel may be attributed to its ability to promote angiogenesis, enhance fibroblast activity and recruit stem cells, thus addressing both the inflammatory and reparative phases of healing[15,16].

The study also highlights the gel's anti-inflammatory properties, as evidenced by the upregulation of CD163 mRNA expression and the downregulation of NF-κB p50 mRNA. The CD163, a marker of M2 macrophages involved in tissue repair, was upregulated, indicating a shift towards an anti-inflammatory environment that facilitates wound healing[17]. The CD163, a marker for M2 macrophages, is involved in tissue repair and inflammation resolution[18]. The gel appears to shift macrophage polarization towards the M2 phenotype, creating a more favorable wound-healing environment[19]. This shift from pro-inflammatory M1 macrophages, common in diabetic foot ulcers, to anti-inflammatory M2 macrophages may break the chronic inflammation cycle, promoting tissue repair and regeneration[20,21].

Interestingly, while the change in wound volume showed a significant negative correlation with CD163 mRNA expression, NF-κB p50 mRNA expression did not exhibit a significant correlation with wound volume changes. However, the negative correlation of the study CD163 and NF-κB p50 suggests a potential interaction of these two proteins in regulating wound healing. The exact mechanism behind this relationship remains to be explored, but CD163 may act as a negative regulator of NF-κB p50 activity, contributing to the resolution of inflammation. The findings of this study are consistent with the results of the recent meta-analysis by Sun *et al.*[22], which evaluated the effectiveness and safety of stem cell therapy for diabetic foot ulcers. The meta-analysis confirmed that stem cell therapy, including the use of SH-MSC gel, significantly enhances the healing of diabetic foot ulcers and has a favorable safety profile. The reduction in wound volume and molecular changes observed in the present study support the evidence that this therapy can effectively address the challenges of diabetic foot ulcer healing, aligning with the conclusions of the meta-analysis.

Despite these promising findings, the study has limitations, including a small sample size, a short study duration of 7 days and a lack of diversity in the patient population. Additionally, the underlying mechanisms of SH-MSC gel's action in this study are not fully explored. Future research should address these limitations by including larger and more diverse patient populations, extending the study duration and investigating the biological pathways involved to gain a more comprehensive understanding of SH-MSC gel's therapeutic potential in treating DFUs.

CONCLUSION

The significant modulation of CD163 and NF-κB p50 mRNA expression by SH-MSC gel underscores its potential to address the multifaceted pathomechanisms of diabetic foot ulcers. The SH-MSC gel offers a promising therapeutic strategy to enhance wound healing in diabetic patients by promoting anti-inflammatory macrophage activity, reducing pro-inflammatory signaling and decreasing wound volume. Understanding these molecular mechanisms provides a strong foundation for further research and development of secretome-based therapies for chronic wounds.

SIGNIFICANCE STATEMENT

This study investigates the therapeutic potential of human mesenchymal stem cell (MSC) gel secretome in enhancing the healing of diabetic foot ulcers (DFUs), a

common and severe complication of diabetes with limited effective treatments. Through a randomized clinical trial, the research explores how MSC gel secretome promotes healing by modulating key inflammatory markers, specifically upregulating NF-κB p50 and CD163 mRNA expression. The results demonstrate a significant improvement in wound closure and inflammation reduction compared to standard treatments, indicating that MSC gel secretome may offer a novel, effective approach to DFU management. This study contributes to expanding the understanding of regenerative medicine by identifying MSC gel secretome as a potential intervention for enhancing wound healing in chronic diabetic conditions.

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