Indonesian Journal of Medical Laboratory Science and Technology
Open Access
p-ISSN 2684-6748
e-ISSN 2656-9825

RESEARCH ARTICLE

Vitamin D enhances migration but decreases gene expression of vascular endothelial growth factor and tumor necrosis factor-α in Wharton's jelly mesenchymal stem cells

Vitriyanna Mutiara Yuhendri [1], Nurfi Pratiwi [1,2], Ariza Julia Paulina [1,3], Ismawati Ismawati [4], Nicko Pisceski Kusika Saputra [5], Arfianti Arfianti [6]

[1]Master of Biomedical Science Program, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia
[2]Department of Histology, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia
[3]Department of Clinical Pathology, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia
[4]Department of Biochemistry, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia
[5]Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia
[6]Department of Medical Biology, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia

Correspondence:
Arfianti
Department of Medical Biology, Faculty of Medicine, Universitas Riau, Pekanbaru, 28133, Indonesia

Email: evi_anti@yahoo.com

Article history:
Received: 2024-11-28
Revised: 2025-02-28
Accepted: 2025-03-17
Available online: 2025-04-22

Keywords:
Interleukin-6
Mesenchymal stem cells
Tumor necrosis factor-α
Vascular endothelial growth factor
Vitamin D

https://doi.org/10.33086/ijmlst.v7i1.6698

Abstract

Mesenchymal stem cells (MSCs) have gained significant interest for their potential application in repairing damaged tissues through immunomodulatory functions. Vitamin D has been shown to regulate both the innate and adaptive immune systems. Active forms of Vitamin D, such as 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) are known to influence the action of mesenchymal stem cells (MSCs) in several mechanisms, such as stimulating their proliferation and osteogenic differentiation, as well as tissue regeneration. This study aimed to investigate the effects of vitamin D supplementation on the biological properties and expression of cytokines and growth factor genes in MSCs isolated from the human umbilical cord (UC). MSCs were isolated from Wharton's jelly (WJ-MSC) of UC, cultured, and supplemented with various concentrations of vitamin D_3. The Cell Counting Kit-8 (CCK-8) assay was used to measure cell viability, and a scratch wound healing assay was conducted to evaluate the migration capacity of MSCs. The mRNA expression levels of vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF)-α, and interleukin (IL)-6 were quantified using reverse transcription-polymerase chain reaction (RT-PCR). This study showed that supplementation with 50 nM of vitamin D_3 for 48 h significantly increased the viability and migratory capacity of MSCs. Furthermore, vitamin D supplementation significantly decreased the mRNA levels of TNF-α and VEGF but did not affect IL-6 gene expression compared to the control group. These findings suggest that vitamin D supplementation can enhance the biological characteristics and modulate the expression of key immunomodulatory factors in MSCs, potentially improving the effectiveness of MSC-based therapies.

1. INTRODUCTION

Regenerative medicine has recently attracted significant interest due to its potential to repair damaged tissues and organs. Mesenchymal stem cells (MSCs) have emerged as a promising resource for tissue repair due to their self-renewal ability, potential for multilineage differentiation, and immunoregulatory properties (1). These multipotent progenitor cells can be obtained from various adult and fetal tissues, including the placenta, umbilical cord, bone marrow, adipose tissue, and dental pulp (2). Fetal-derived MSCs demonstrate a higher proliferation rate and can undergo more passages in vitro before senescence than MSCs obtained from adult tissues. However, umbilical cord mesenchymal stem cells (UC MSCs) offer

Citation: Yuhendri VM, Pratiwi N, Paulina AJ, Ismawati I, Saputra NPK, Arfianti A. Vitamin D enhances migration but decreases gene expression of vascular endothelial growth factor and tumor necrosis factor-α in Wharton's jelly mesenchymal stem cells. Indones J Med Lab Sci Technol. 2025;7(1):49–59. https://doi.org/10.33086/ijmlst.v7i1.6698

several advantages over other MSC sources, including their non-invasive tissue collection, ethical acceptability, and most notably, their high proliferation rate, which significantly enhances their practicality for clinical application (3–5).

Mesenchymal stem cells produce a secretome of bioactive molecules, such as growth factors, chemokines, cytokines, proteins, lipids, and miRNA (6). Increasing evidence suggests that the secretome derived from MSCs could significantly impacts the positive effects of MSCs by influencing the cellular immune response and increasing the regenerative capacity of resident cells within the injured tissue (7–9). In particular, the ability of MSCs to regulate the immune response has been linked to the release of Tumour Necrosis Factor alpha (TNF-α), Interleukin (IL)-6, and Vascular Endothelial Growth Factor (VEGF) (10,11). In addition to having previously described therapeutic properties, MSCs must migrate to damaged tissues to be involved in regeneration. The migration of MSCs into injured tissues after transplantation is a complex and intriguing process that depends on various mechanical and chemical factors (12).

Despite the promising evidence from preclinical studies, the therapeutic efficacy of MSCs in clinical studies remains limited. The translation of MSCs-based therapies into clinical practices is challenging with issues such as low survival, engraftment, limited migration capacity, and increased cellular senescence of MSCs (13). To address these challenges, it is crucial to focus on reducing cellular senescence and increasing cellular viability, proliferation, and migration capacity. Various strategies have been explored to achieve these goals. Furthermore, preconditioning methods like hypoxia, cytokine priming, three-dimensional (3D) cell culture, genetic modification, and bioactive compounds have been employed to enhance the immunomodulatory effects of MSCs (14,15).

Vitamin D, a pivotal pro-hormone, regulates a multitude of physiological processes, from bone metabolism to immune regulation, and cellular differentiation (16). Notably, previous studies have shown that vitamin D can modulate the immune system (17) and influence the differentiation and proliferation of adipose- and bone marrow-derived MSCs (18). The most recent evidence suggests a compelling potential, vitamin D may significantly affect the therapeutic efficacy of MSCs (19), a discovery that piques curiosity and invites further investigation.

Despite the promising preclinical evidence, the therapeutic efficacy of MSC in clinical applications remains limited due to challenges such as low survival rates, limited migration capacity, and increased cellular senescence. While various strategies, including preconditioning approaches like hypoxia and genetic modifications, have been explored, the potential of vitamin D supplementation as a priming agent for MSCs remains under investigation. Previous studies have primarily focused on the effects of vitamin D on MSCs derived from adipose or bone marrow tissues, leaving a significant knowledge gap regarding its impact on umbilical cord-derived MSCs (UC-MSCs). These UC-MSCs, with their unique biological advantages, are a fascinating area of study. This study aims to fill the gap by examining the effects of vitamin D supplementation on the viability, migratory capacity, and gene expression of key immunomodulatory factors in UC-MSCs. The novelty of this study lies in its focus on utilizing vitamin D as a supplementation agent to enhance the therapeutic potential of UC-MSCs, offering new insights into the optimization of MSC-based therapies.

2. MATERIALS AND METHODS

2.1. Isolation and Expansion of WJ-MSC

Wharton's jelly (WJ) from the umbilical cord (UC) of cesarean mothers aged 20–35 years was used as the source of MSCs (WJ-MSC). All participants (cesarean mothers) provided informed consent before umbilical cord donation. The WJ MSCs were isolated and cultured with the utmost care and precision using the explant method (20) as previously published (21) with a few modifications. Briefly, the UC was transversely incised to a length of 5 cm. Wharton's jelly was separated from the UC, both umbilical arteries and veins were removed, and the WJ was cut into small pieces. The WJ fragments were then placed in a 100 mm diameter culture dish (Corning, New York, USA) containing a culture medium composed of Dulbecco's Modified Eagle's Medium (DMEM) (Corning, New York, USA) supplemented with 5% human platelet lysate (HPL), 3 IU heparin, 1% Glutamax, and 1% penicillin-streptomycin. The MSCs were incubated in a humidified incubator at 37°C with 5% CO_2. The culture medium was changed every 2–3 days, and cells were split when they reached 80–90% confluence using 0.25% trypsin-EDTA (Sigma-Aldrich, Darmstadt, Germany). The WJ-MSC used in these experiments were in passages 3–5.

2.2. Cell Viability Assay

The WJ-MSC were seeded at a $5.0x10^4$ cells/cm^2 density on a 96-well culture plate. The cells were then cultured in DMEM supplemented with vitamin D_3 (cholecalciferol) (Sigma-Aldrich, Taufkirchen, Germany), at 0–100 concentrations nM for 24 and 48 h. The viability of WJ-MSC was thoroughly examined using the Cell Counting Kit-8 assay (CCK-8) (ABBKINE, Wuhan, China), according to the manufacturer's protocols. Wells without cells were used as blanks. The absorbance was measured at a wavelength of 450 nm. The percentage of cell viability was calculated using the formula 1 where ODs represent the optical density of the sample, ODb represents the optical density of the blank, and ODc represents the optical density of the control, where the control refers to WJ-MSC cultured in media without the addition of vitamin D_3.

$$\text{Cell Viability (\%)} = \frac{\text{ODs}-\text{ODb}}{\text{ODc}-\text{ODb}} \times 100\% \quad \text{..... (1)}$$

2.3. Migration Assay

The WJ-MSC were seeded in a six-well culture plate ($5.0x10^4$ cells/cm^2) for migration assay. After overnight incubation or cells reached 70-80% confluence, the old medium was replaced with DMEM containing vitamin D_3. The migration capacity of WJ-MSC was assessed using the scratch wound healing assay. The scratched cell-free zone was manually made across the cell monolayer using a sterile 1000 µL pipette tip. The cells were then washed off twice with Dulbecco's Phosphate Buffered Saline (DPBS) (Sigma-Aldrich, Taufkirchen, Germany) to remove cellular debris. Subsequently, a culture medium containing 50 nM of vitamin D_3 was added, and cells were cultured for 24 and 48 hours. The migration of WJ-MSC was observed and photographed using an inverted microscope (Olympus CKX53, Olympus, Tokyo, Japan). The wound area was calculated with the reliable ImageJ software, ensuring the accuracy of the results. The wound closure percentage was obtained using the following formula 2, where T0 was the wound area at 0 h and Tt was the remaining area at the designated time (22).

$$\text{Wound Closure Rate } (\%) = \frac{(T0-Tt)}{T0} \times 100\% \quad (2)$$

2.4. Real-Time Quantitative PCR

The WJ-MSC used for Real-time quantitative PCR (RT-qPCR) experiments were seeded in six-well culture plates ($5.0x10^4$ cells/cm^2). The cells were cultured in a medium supplemented with vitamin D_3 at a concentration of 50 nM and incubated for 48 h in a CO_2 incubator. Total RNA was extracted using TRNzol Universal Reagent (Tiangen Biotech, Beijing, China), according to the manufacturer's instructions. RNA concentration and purity were examined using a Multiskan SkyHigh Microplate Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). A total of 1 microgram RNA was transcribed into cDNA using the GoTaq® 2-Step RT-qPCR system (Promega Corporation, Madison, WI, USA). The targeted genes were amplified using primer sequences shown in Table 1 (23–25) on Heal Force Real-Time PCR X 960 under the following conditions (polymerase activation at 95°C, denaturation at 95°C, and annealing at 60°C). Real-time quantitative PCR (RT qPCR) was performed using GoTaq® 2-Step RT-qPCR. The gene expression levels were compared using the robust and reliable relative quantification ($2^{-\Delta\Delta Ct}$) method, with. GAPDH serving as an internal reference to normalize the mRNA expression levels. In addition, melting curve analysis was performed to determine the specificity of the PCR products.

Table 1. qPCR Primer Sequences

Gene	Forward	Reverse	Reference
GAPDH	5'-GACCTGCCGTCTAGAAAAAC-3'	5'-TTGAAGTCAGAGGAGACCAC-3'	(23)
TNF- α	5'-TGGCCAATGGCGTGGAGCTG-3'	5'-GTAGGAGACGGCGATGCGGC-3'	Designed using the Primer-BLAST
VEGF-A	5'-ATCTGCATGGTGATGTTGGA-3'	5'-GGGCAGAATCATCAC GAAG-3'	(24)
IL-6	5'-AAGCCAGAGCTGTGCAGATGAGTA-3'	5'-TGTCCTGCAGCCACTGGTTC-3'	(25)

2.5. Statistical Analysis

The experimental data, expressed as the mean ± standard deviation (SD), were analyzed using the reliable GraphPad Prism v 9. We applied the One-way ANOVA test and Tukey's multiple comparisons test to compare the cell viability between experimental groups. The effects of vitamin D supplementation on the migration analysis were examined using two-way ANOVA, and the effects of vitamin D supplementation on mRNA expression of IL-6, TNF-α, and VEGF were analyzed using an unpaired t-test Statistical significance was determined if p-value<0.05, further reinforcing the accuracy of our conclusions.

3. RESULTS AND DISCUSSION

3.1. Effect of Vitamin D on The Viability of WJ-MSCs

The viability of WJ-MSC following supplementation with various vitamin D concentrations for 24 and 48 h was examined using the CCK-8 Assay. After 24 h of vitamin D_3 supplementation at 1 nM and 50 nM, viability increased. However, cell viability tended to decrease at higher concentrations of 100 nM (Figure 1). Despite these changes, the statistical test did not identify significant differences between the experimental groups ($p > 0.05$). After 48 hours of vitamin D_3 supplementation at 1–100 nM concentrations, a significant increase in WJ-MSC viability was observed at 50 nM compared to the control group ($p = 0.0437$ and $p = 0.0091$, respectively) (Figure 2). Notably, prolonged incubation with 50 nM of vitamin D_3 for 48 hours resulted in a significant increase in WJ-MSC viability. These results underscore the significant

impact of vitamin D_3 on WJ-MSC viability and provide a solid foundation for further analysis of cell migration and the expression of TNF-α, IL-6, and VEGF.

The effects of vitamin D on MSC survival vary depending on the tissue source of MSCs, species of origin, and the concentration of vitamin D (26). Consistent with our study, adding calcitriol (vitamin D) at concentrations exceeding 50 nM was cytotoxic to adipose-derived MSCs (27,28). Similarly, their viability decreases when dental pulp-derived MSCs were treated with vitamin D at concentrations ranging from 1 to 100 nM (27). However, bone marrow-derived MSCs (BM-MSCs) did not significantly impact cell viability at the same concentrations of vitamin D (28), even though their osteogenic differentiation capacity increased. In contrast, 20 nM and 40 nM of vitamin D_3, even for more extended incubation periods of 1–5 d, significantly increased the viability of BM-MSCs (27). Therefore, our study and other studies' results indicate that lower concentrations of vitamin D (less than 100 nM) do not have a toxic effect on MSCs.

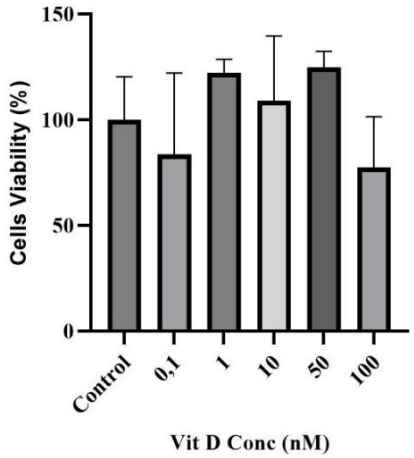

Figure 1. The effect of vitamin D_3 supplementation for 24 h on the viability of Wharton's jelly -derived mesenchymal stem cells. Cell viability was measured using the cell counting kit-8 (CCK-8) assay. Data are presented as mean ± SD (n=5)

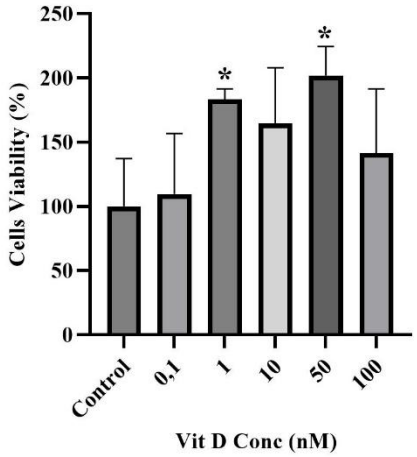

Figure 2. The effect of vitamin D_3 supplementation for 48 h on the viability of Wharton's jelly-derived mesenchymal stem cells. Cell viability was measured using the CCK-8 assay. Data are presented as mean ± SD (n=5). *p<0.05 compared to controls using one-way ANOVA and post hoc Tukey's multiple comparison analysis.

Vitamin D may have a profound impact on the cellular viability of MSCs through several mechanisms. One proposed pathways is sirtuin 1 (SIRT1) signaling, which has been shown to enhance MSC proliferation and maintain stemness by upregulating pluripotency markers (26). Although Vitamin D has been reported to induce osteogenic differentiation in adipose-derived MSCs via BMP2 signaling (29), the direct link between this pathway and MSC viability remains unclear. This potential for Vitamin D to affect MSC survival or highlights an exciting area for further research in the field of regenerative medicine. Further studies are needed to determine whether the observed differentiation enhances MSC survival or simply alters their cellular function.

In addition to its stimulatory effects on cell viability, vitamin D promotes osteoblastic differentiation of MSC isolated from dental tissues (30) and alveolar periosteum (31). Several mechanisms are involved in how vitamin D increases the differentiation capacity of MSCs, including enhancing the expression of RUNX2 (a member of the PEBP2/CBF transcription factors family controlling the expression of genes whose products are essential for bone formation) and collagen I, leading to increased production of mineralized matrix nodules (30). Furthermore,

vitamin D upregulates specific osteogenic and adipogenic genes (32), which are crucial for the differentiation process, and stimulates MSCs adhesion by increasing Alpha-v beta-3 (αvβ3) integrin expression and focal adhesion formation, which is crucial for cell commitment and differentiation (33).

The active form of vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) also known as calcitriol, has been found to influence multiple cellular signaling pathways that impact cell viability. This vitamin is crucial role in balancing autophagy and apoptosis, which is fundamental for cell survival. It enhances the expression of pro-autophagic proteins like Beclin 1 and induces the phosphorylation of Extracellular signal-regulated kinase 1/2 (ERK1/2) and Akt, components of survival signaling pathways (34). Consequently, this reduces of apoptosis-related gene expression and helps maintain mitochondrial function. Moreover, the interaction between vitamin D_3-up-regulated protein-1 and thioredoxin, a critical element in redox signaling, regulates cardiomyocyte viability under stress conditions (35). This interaction is essential for managing oxidative stress and preventing apoptosis. Additionally, vitamin D interacts with the TGF-beta signaling pathway via SMAD proteins, which is significant in regulating cell proliferation and differentiation (36). Furthermore, recent studies have demonstrated that vitamin D_3 can significantly attenuate H_2O_2-induced cell injury in MSCs through the Sirt1/FoxO1 signaling pathway. This protective effect is achieved by reducing intracellular oxidative stress caused by H_2O_2 exposure, thereby enhancing the resilience of MSCs under oxidative conditions. These findings further emphasize the crucial role of vitamin D in cellular defense mechanisms and its potential therapeutic applications in regenerative medicine (37).

3.2. Effect of Vitamin D on Migration of WJ-MSCs

The migration capacity of WJ-MSC supplemented with vitamin D was assessed using the scratch wound healing assay. Images were captured at fixed intervals of 0, 24, and 48 h (Figure 3). A noticeable gap in the control group remained in the scratched region at 24 and 48 h. In contrast, the scratched area began to close in WJ-MSC administered with 50 nM vitamin D_3 for 24 h (Figure 3D), and there was no visible space at 48 h (Figure 3F). Figure 3G shows the percentage of wound closure in the WJ-MSC group. Exposure to vitamin D_3 for 24 h significantly increased the percentage of wound closure in WJ-MSCs compared with the control group (33.7% vs 55.12%, p=0.0178). Similarly, after 48 h, a highly significant difference was observed in the percentage of closure area between WJ-MSC supplemented with 50 nM vitamin D_3 and control WJ-MSC (88.67% vs. 49.36%, p=0.0020). These data demonstrate that vitamin D_3 supplementation increased the wound closure rate of WJ-MSC, indicating that vitamin D_3 enhances the migratory ability of WJ-MSC. To the best of our knowledge, this is the first study to investigate the effects of vitamin D on the migration of MSCs.

Vitamin D has been demonstrated to enhance the migration rates of various cell types. For instance, calcipotriol, a synthetic analog of vitamin D_3, has been shown to increase the rate of wound closure in keratinocyte cells (38). Additionally, vitamin D has been found to promote migration and inhibit apoptosis of vascular smooth muscle cells (VSMCs) obtained from rats (39). Human umbilical vein endothelial cells (HUVECs) cultured in a 3-dimensional matrix and supplemented with vitamin D exhibited increased proliferation and migration capacity, which was associated with increasing the expression of matrix metalloproteinase-2 (MMP-2) (40). Furthermore, vitamin D_3 has been shown to enhance the promoting effects of nano hydroxyapatite composite scaffolds on the osteogenic differentiation capacity of human adipose-MSCs (41), inspiring new avenues for bone regeneration research.

The capacity to migrate and home to damaged tissues is critical to the ability of MSCs to repair damaged tissue (42). Various factors, including chemical signals, growth factors, and environmental conditions, influence the migration of mesenchymal stem cells (MSCs). These factors play crucial roles in guiding MSCs to sites of injury or disease, which is crucial for their therapeutic efficacy. Chemotactic agents such as stromal cell-derived factor-1 (SDF-1), transforming growth factor-beta (TGF-β), and substance P (SP) play pivotal roles in regulating MSC mobilization and movement, often interacting in complex ways. Furthermore, MSCs secrete matrix metalloproteinase 1 (MMP-1), which plays a role in facilitating MSC movement by activating protease-activated receptor 1 (PAR1). The production of MMP-1 is amplified by inflammatory mediators such as IL-1β (43,44). In addition to these internal factors, chemokines, growth factors, and inflammatory mediators in the surrounding environment can profoundly influence MSC migration by triggering specific signaling pathways. To the best of our knowledge, there is no direct evidence regarding the effects of vitamin D on the aforementioned factors associated with the migration of MSCs. Therefore, the urgency and significance of further studies to confirm the effect of vitamin D on the migration capacity of WJ-MSC (45) cannot be overstated.

Vitamin D has been extensively studied for its inhibitory effect on cancer cell migration. An in vitro study demonstrated that 1 μM of vitamin D inhibited the migration of thyroid cancer cells (46) and ovarian cancer cells (47). The latter study also found that the inhibitory effect of vitamin D on cancer cell growth was mediated through the upregulation of the galectin-3 (GAL-3) gene expression (47). One study examined the effects of vitamin D supplementation on human umbilical vein endothelial cells (HUVECs) subjected to high glucose levels. Vitamin D decreased apoptosis, increased migration, and enhanced the viability of HUVECs exposed to high glucose (48). These findings underscore Vitamin D's complex and multifaceted role in cellular processes, highlighting its potential significance in cancer therapeutics and MSC biological properties.

3.3. Effect of Vitamin D Supplementation on The Gene Expression Levels of TNF-α, IL-6, and VEGF

The gene expression levels of TNF-α, IL-6, and VEGF in WJ-MSC after vitamin D_3 administration were examined using RT qPCR for mRNA expression analysis (Figure 4). The gene expression was normalized to that of GAPDH, which was used as the reference control. Figure 4A shows a significant decrease in TNF-α expression in WJ-MSC treated with 50 nM vitamin D_3 compared with the control group (p=0.0435). Similarly, treatment with 50 nM vitamin D_3 for 48 h reduced the mRNA expression of VEGF in WJ-MSC compared to those without vitamin D_3 supplementation (Figure 4B, p=0.0197). However, administration of 50 nM vitamin D_3 for 48 h did not affect the mRNA expression of IL-6 in WJ-MSC compared to the control group (Figure 4C, p=0.5538). These findings have significant implications for the potential therapeutic applications of vitamin D_3 supplementation.

The immunomodulatory effects of MSCs hold significant promise for their therapeutic potential in managing pathological conditions associated with immune responses. These conditions include autoimmune diseases, chronic inflammation, or organ transplantation. Mesenchymal stem cells have been shown to produce a range of cytokines and growth factors that can regulate immune responses. In the present study, WJ-MSC were found to express TNF-α. However, preconditioning with vitamin D_3 for 48 hours significantly decreased the expression of TNF-α. This finding contrasts with a previous study using human bone marrow-derived MSCs, which did not to detect TNF-α protein expression using the enzyme-linked immunosorbent assay (ELISA) method (49).

Tumor necrosis factor-alpha (TNF-α) is a versatile factor in modulating the therapeutic effects of MSCs by influencing their immunoregulatory and differentiation capacities. TNF-α's interaction with tumor necrosis factor-alpha receptor (TNFR)1 and TNFR2, mediates pro-inflammatory and anti-inflammatory responses, showcasing its dual function (50). This versatility is crucial in MSC-based therapies for autoimmune and inflammatory conditions, with TNFR2 being particularly vital in enhancing the MSCs' immunosuppressive functions, such as stimulating regulatory T cells and suppressing effector T cells (50). Additionally, TNF-α's impacts on MSC differentiation, such as promoting neural differentiation and enhancing migration towards gliomas, presents exciting possibilities for neurodegenerative therapies (51). In inflammatory environments, TNF-α's involvement in the regulation of MSC fate and functional reprogramming significantly influences their capacity for tissue regeneration and repair.

The relationship between vitamin D and VEGF expression in MSCs has not been directly demonstrated in previous studies. However, it is known that the active form of vitamin D, 1,25-dihydroxyvitamin D_3, can directly increase VEGF expression in vascular smooth muscle cells by binding to response elements in the VEGF promoter (52). This suggests that vitamin D may have complex regulatory effects on VEGF expression, depending on the cell type and specific conditions. The observed decrease in VEGF expression in WJ-MSC following vitamin D_3 preconditioning in the present study underscores the intricate nature of this relationship and the need for further investigation into the mechanisms underlying the interaction between vitamin D and growth factor expression in different MSCs.

VEGF promotes blood vessel formation by stimulating the growth of vascular endothelial cells in arteries, veins, and lymphatic vessels (53). Umbilical cord-derived MSCs have been shown to express VEGF, which can stimulate angiogenesis and increase the therapeutic effects of MSC therapy (54). According to our study, vitamin D supplementation significantly decreased the expression of VEGF on WJ-MSC, which contradicts the results of a study that utilized hypoxic conditions in MSCs, increasing VEGF expression (21). These results suggest that different preconditioning treatments may have distinct effects on gene expression despite employing the exact source of MSCs. This difference may be attributed to different sources of the MSCs used in the experiments. Mass spectrometry analysis of conditioned media of MSCs isolated from various tissues revealed that MSCs obtained from fetal tissues, such as the placenta and umbilical cord, secreted more diverse proteins than MSCs from adipose tissue and bone marrow (55,56). Interestingly, despite these differences, MSCs were predicted to exert comparable biological properties, including promotion migration and inhibiting cell apoptosis regardless of their tissue of origin. This may be due to the similar enrichment of proteins involved in the promotion of migration and the reduction of apoptosis in MSC from all sources.

Another cytokine MSCs express is IL-6, a pro-inflammatory cytokine involved in various biological processes. IL-6 is the most abundantly produced cytokine by MSCs derived from human bone marrow (49). IL-6 secreted by MSCs can shift the macrophage phenotypes towards M2 anti-inflammatory (57). Our study showed that vitamin D_3 preconditioning tended to decrease the expression of IL-6, although the difference was not statistically significant. While there is no study on the effect of vitamin D on IL-6 expression in MSCs, a study using stromal vascular cells (SVCs) demonstrated that vitamin D supplementation decreased IL-6 expression (58). Interleukin-6 (IL-6) plays a significant role in the therapeutic effects of mesenchymal stem cells (MSCs) by modulating immune responses and promoting tissue repair. IL-6 secreted by MSCs promotes the polarization of macrophages towards an anti-inflammatory M2 phenotype, which is crucial for reducing inflammation and promoting tissue repair in conditions like peritoneal fibrosis and systemic lupus erythematosus (57,59). In hypoxic-ischemic brain damage, IL-6 from MSCs activates signaling pathways that reduce the proliferation of reactive astrocytes, aiding in functional recovery (60). Given that IL-6 plays a crucial role in the therapeutic effects of MSCs, targeting IL-6 could be a promising approach to improve the effectiveness of MSC-based treatments for various inflammatory and

degenerative disorders. To our knowledge, this is the first study to examine IL-6 expression in MSCs. However, further research is urgently needed to validate and expand upon these findings, underscoring the importance of your work in advancing the field of regenerative medicine.

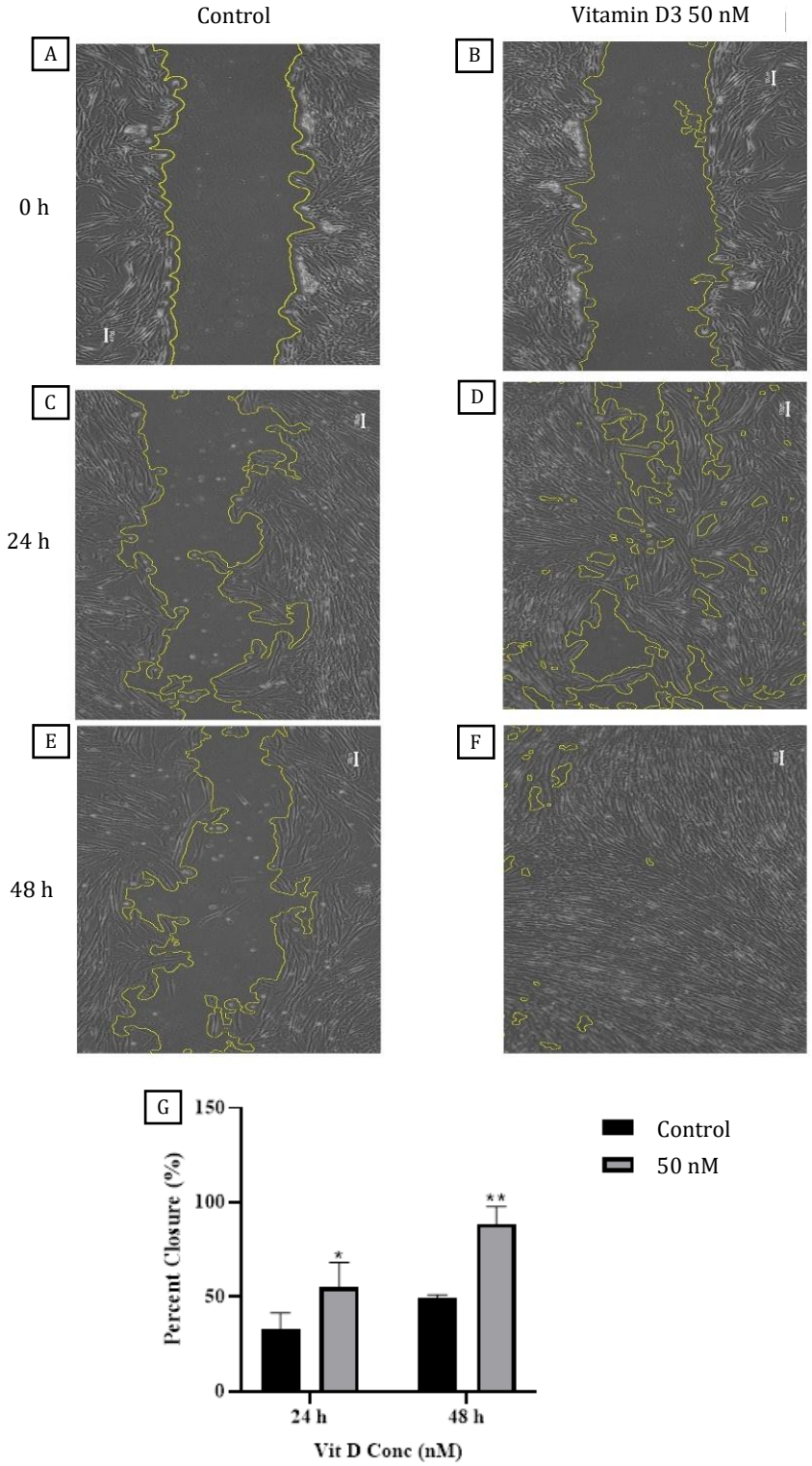

Figure 3. Scratch Wound Healing Assay on Wharton's jelly-derived mesenchymal stem cells. (A-B) WJ-MSC immediately after the scratch was made (T0); (C-D) after 24 h of exposure (T24); (E-F) after 48 h of exposure (T48) (n=3; Magnification 40X). (G) closure percentage after preconditioning with 50 nM vitamin D_3 on WJ-MSCs. Data are presented as mean ± SD and analyzed using two-way ANOVA. *$p < 0.05$ and **$p < 0.01$ compared to the control group. The control refers to WJ-MSC cultured in media without adding vitamin D3.

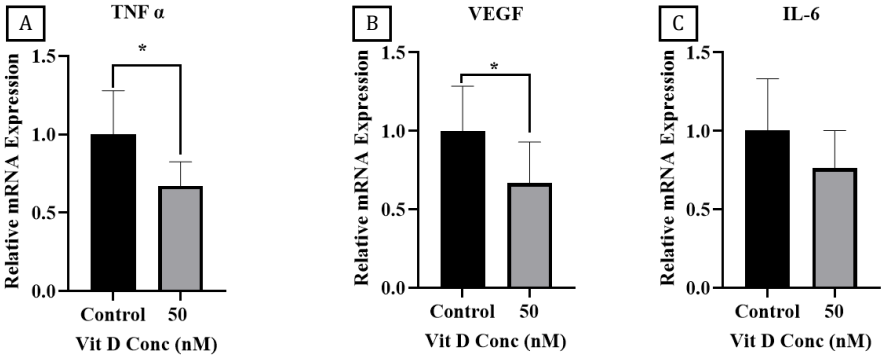

Figure 4. Wharton's jelly-derived mesenchymal stem cells supplementation with vitamin D_3 affects gene expression. RT qPCR analysis was conducted on Wharton's jelly-derived mesenchymal stem cells (WJ-MSC) after 48 h of supplementation with the expression of TNF-α (A) IL-6 (B) and VEGF (C) mRNA was normalized to GAPDH. The data are presented as mean ± SD (n=6). *p<0.05 compared to controls using the t-test. The control refers to WJ-MSC cultured in media without adding vitamin D_3.

Rigorous evaluation of preconditioning effects on MSCs is essential for their successful translation into clinical applications. This evaluation encompasses various aspects of MSC functionality, including survival, migration, proliferation, regeneration potential, and immunoregulatory effects in response to preconditioning or priming strategies. These factors must be thoroughly investigated to ensure the optimal performance of MSCs in therapeutic settings (61,62). The MSCs secretome, which comprises the bioactive molecules secreted by these cells, also plays a crucial role in their regenerative potential. The composition of the secretome is significantly influenced by the tissue of origin, the local microenvironment, and the conditions under which the cells are cultured (6).

Given the importance of the MSCs secretome in determining therapeutic efficacy, it is imperative to explore and develop potential preconditioning or priming strategies that can enhance the production of bioactive molecules by MSCs. These strategies may involve manipulating culture conditions, exposing MSCs to specific stimuli, or modifying their genetic makeup to optimize their secretory profile (63,64). By boosting the production of beneficial factors, such as growth factors, cytokines, and extracellular vesicles, these approaches may augment the overall therapeutic efficacy of MSCs. However, in this study, we focused on evaluating gene expression changes following vitamin D_3 preconditioning and did not perform functional assays to validate these findings further. It is important to note that future studies incorporating functional validation will be necessary to confirm the biological significance of these molecular changes in MSC-based therapies.

4. CONCLUSIONS

In conclusion, the study's findings highlight the potential of vitamin D supplementation to significantly enhance the functionality and therapeutic efficacy of on WJ-MSC. The observed increase in cell viability and migration capacity at a 50 nM concentration of vitamin D suggests potential enhancements in these cells' overall functionality and therapeutic efficacy. Moreover, the modulation of TNF-α and VEGF expression indicates that vitamin D may play a crucial role in regulating the immunomodulatory properties of WJ-MSC, which are essential for their therapeutic applications in various diseases and conditions.

These results provide compelling evidence for using vitamin D_3 as a supplementation strategy to enhance the therapeutic capacity of MSCs. However, it is important to note that while these findings are promising, they are primarily based on in vitro studies. Further research is necessary to fully elucidate the mechanisms underlying vitamin D's effects on MSCs and to determine whether these benefits translate to improved clinical outcomes when vitamin D-supplemented MSCs are administered to patients. This research is crucial to assess the long-term safety and efficacy of this approach and optimize the dosage and timing of vitamin D supplementation for maximum therapeutic benefit.

Author contributions: AA: Conceptualization, methodology, validation, data curation, supervision. AA, VMY: Software, writing—review and editing. AA, NP, AJP: Formal analysis. II: Investigation. NPKS: Resources. AA, VMY, NP, AJP, II, NPKS: Writing—original draft preparation. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Ministry of Education, Culture, Research, and Technology, Directorate of Research, Technology, and Community 2024 (Contract No. 20588/UN19.5.1.3/AL.04/2024 and 20713/UN19.5.1.3/AL.04/2024.

Acknowledgements: We would like to thank the medical staff of the Department of Obstetrics and Gynecology, Hermina Hospital for their valuable contributions in obtaining umbilical cord samples.

Ethics statement: This research was approved by the Research Etichs Committee of Faculty of Medicen Universitas Riau with No: B/ 047/ UN19.5.1.1.8/UEPKK/2021_Adendum2.

Conflict of interest: The authors have no conflict of interest to disclose.

REFERENCES

1. Margiana R, Markov A, Zekiy AO, Hamza MU, Al-Dabbagh KA, Al-Zubaidi SH, et al. Clinical application of mesenchymal stem cell in regenerative medicine: A narrative review. Stem Cell Res Ther. 2022;13(1):366. https://doi.org/10.1186/s13287-022-03054-0
2. Mebarki M, Abadie C, Larghero J, Cras A. Human umbilical cord-derived mesenchymal stem/stromal cells: A promising candidate for the development of advanced therapy medicinal products. Stem Cell Res Ther. 2021;12(1):152. https://doi.org/10.1186/s13287-021-02222-y
3. Song I, Rim J, Lee J, Jang I, Jung B, Kim K, et al. Therapeutic potential of human fetal mesenchymal stem cells in musculoskeletal disorders: A narrative review. Int J Mol Sci. 2022;23(3):1439. https://doi.org/10.3390/ijms23031439
4. Jovic D, Yu Y, Wang D, Wang K, Li H, Xu F, et al. A brief overview of global trends in msc-based cell therapy. Stem Cell Rev Rep. 2022;18(5):1525–45. https://doi.org/10.1007/s12015-022-10369-1
5. Guenther R, Dreschers S, Maassen J, Reibert D, Skazik-Voogt C, Gutermuth A. The treasury of Wharton's jelly. Stem Cell Rev Rep. 2022;18(5):1627–38. https://doi.org/10.1007/s12015-021-10217-8
6. Trigo CM, Rodrigues JS, Camões SP, Solá S, Miranda JP. Mesenchymal stem cell secretome for regenerative medicine: Where do we stand?. J Adv Res. 2024;S2090-1232(24)00181-4. https://doi.org/10.1016/j.jare.2024.05.004
7. Jimenez-Puerta GJ, Marchal JA, López-Ruiz E, Gálvez-Martín P. Role of mesenchymal stromal cells as therapeutic agents: Potential mechanisms of action and implications in their clinical use. JCM. J Clin Med. 2020;9(2):445. https://doi.org/10.3390/jcm9020445
8. Navarro LVL, Chen X, Viviescas LTG, Ardila-Roa AK, Luna-Gonzalez ML, Sossa CL, et al. Mesenchymal stem cells for critical limb ischemia: Their function, mechanism, and therapeutic potential. Stem Cell Res Ther. 2022;13(1):345. https://doi.org/10.1186/s13287-022-03043-3
9. Huang Y, Wu Q, Tam PKH. Immunomodulatory mechanisms of mesenchymal stem cells and their potential clinical applications. Int J Mol Sci. 2022;23(17):10023. https://doi.org/10.3390/ijms231710023
10. Eleuteri S, Fierabracci A. Insights into the Secretome of mesenchymal stem cells and its potential applications. Int J Mol Sci. 2019;20(18):4597. https://doi.org/10.3390/ijms20184597
11. Kehl D, Generali M, Mallone A, Heller M, Uldry A-C, Cheng P, et al. Proteomic analysis of human mesenchymal stromal cell secretomes: a systematic comparison of the angiogenic potential. NPJ Regen Med. 2019;4:8. https://doi.org/10.1038/s41536-019-0070-y
12. Fu X, Liu G, Halim A, Ju Y, Luo Q, Song G. Mesenchymal stem cell migration and tissue repair. Cells. 2019;8(8):784. https://doi.org/10.3390/cells8080784
13. Mastrolia I, Foppiani EM, Murgia A, Candini O, Samarelli AV, Grisendi G, et al. Challenges in clinical development of mesenchymal stromal/stem cells: Concise review. Stem Cells Transl Med. 2019;8(11):1135–48. https://doi.org/10.1002/sctm.19-0044
14. Uberti B, Plaza A, Henríquez C. Pre-conditioning strategies for mesenchymal stromal/stem cells in inflammatory conditions of livestock species. Front Vet Sci. 2022;9:806069. https://doi.org/10.3389/fvets.2022.806069
15. Su Y, Xu C, Cheng W, Zhao Y, Sui L, Zhao Y. Pretreated mesenchymal stem cells and their secretome: Enhanced immunotherapeutic strategies. Int J Mol Sci. 2023;24(2):1277. https://doi.org/10.3390/ijms24021277
16. Charoenngam N, Shirvani A, Holick MF. Vitamin D for skeletal and non-skeletal health: What we should know. J Clin Orthop Trauma. 2019;10(6):1082–93. https://doi.org/10.1016/j.jcot.2019.07.004
17. Athanassiou L, Mavragani CP, Koutsilieris M. The immunomodulatory properties of vitamin D. Mediterr J Rheumatol. 2022;33(1):7–13. https://doi.org/10.31138/mjr.33.1.7
18. Fernández-Barral A, Bustamante-Madrid P, Ferrer-Mayorga G, Barbáchano A, Larriba MJ, Muñoz A. Vitamin D effects on cell differentiation and stemness in cancer. Cancers (Basel). 2020;12(9):2413. https://doi.org/10.3390/cancers12092413
19. Hou Y-C, Lu C-L, Zheng C-M, Liu W-C, Yen T-H, Chen R-M, et al. The role of vitamin D in modulating mesenchymal stem cells and endothelial progenitor cells for vascular calcification. Int J Mol Sci. 2020;21(7):2466. https://doi.org/10.3390/ijms21072466
20. Todtenhaupt P, Franken LA, Groene SG, van Hoolwerff M, van der Meeren LE, van Klink JMM, et al. A robust and standardized method to isolate and expand mesenchymal stromal cells from human umbilical cord. Cytotherapy. 2023;25(10):1057–68. https://doi.org/10.1016/j.jcyt.2023.07.004
21. Arfianti A, Ulfah, Hutabarat LS, Agnes IG, Budiarti AD, Sahara NS, et al. Hipoxia modulates the secretion of growth factors of human umbilical cord-derived mesenchymal stem cells. Biomedicine (Taipei). 2023;13(3):49–56. https://doi.org/10.37796/2211-8039.1416
22. Chen J, Liu Y, Zhang J, Yang Y, Liang H, Li T, et al. External application of human umbilical cord-derived mesenchymal stem cells in hyaluronic acid gel repairs foot wounds of types I and II diabetic rats through paracrine action mode. Stem Cells Transl Med. 2023;12(10):689-706. https://doi.org/10.1093/stcltm/szad050
23. Xiang D, Zou J, Zhu X, Chen X, Luo J, Kong L, et al. Physalin D attenuates hepatic stellate cell activation and liver fibrosis by blocking TGF-β/Smad and YAP signaling. Phytomedicine. 2020;78:153294. https://doi.org/10.1016/j.phymed.2020.153294
24. Mathot F, Rbia N, Thaler R, Dietz A, Van Wijnen A, Bishop A, et al. Gene expression profiles of human adipose-derived mesenchymal stem cells dynamically seeded on clinically

available processed nerve allografts and collagen nerve guides. Neural Regen Res. 2021;16(8):1613. https://doi.org/10.4103/1673-5374.303031

25. Wang D, Wang S, Huang S, Zhang Z, Yuan X, Feng X, et al. Serum IFN-γ predicts the therapeutic effect of mesenchymal stem cells transplantation in systemic lupus erythematosus patients. Stem Cells Transl Med. 2017;6(9):1777–85. https://doi.org/10.1002/sctm.17-0002

26. Borojević A, Jauković A, Kukolj T, Mojsilović S, Obradović H, Trivanović D, et al. Vitamin D_3 stimulates proliferation capacity, expression of pluripotency markers, and osteogenesis of human bone marrow mesenchymal stromal/stem cells, partly through SIRT1 signaling. Biomolecules. 2022;12(2):323. https://doi.org/10.3390/biom12020323

27. Escobar LM, Bendahan Z, Bayona A, Castellanos JE, González M-C. Effect of vitamins D and E on the proliferation, viability, and differentiation of human dental pulp stem cells: An in vitro study. Int J Dent. 2020;2020:8860840. https://doi.org/10.1155/2020/8860840

28. Lee H-J, Song Y-M, Baek S, Park Y-H, Park J-B. Vitamin D enhanced the osteogenic differentiation of cell spheroids composed of bone marrow stem cells. Medicina (Kaunas). 2021;57(11):1271. https://doi.org/10.3390/medicina57111271

29. Song I, Kim B-S, Kim C-S, Im G-I. Effects of BMP-2 and vitamin D_3 on the osteogenic differentiation of adipose stem cells. Biochem Biophys Res Commun. 2011;408(1):126–31. https://doi.org/10.3390/medicina57111271

30. Posa F, Di Benedetto A, Colaianni G, Cavalcanti-Adam EA, Brunetti G, Porro C, et al. Vitamin D effects on osteoblastic differentiation of mesenchymal stem cells from dental tissues. Stem Cells Int. 2016;2016:9150819. https://doi.org/10.1155/2016/9150819

31. Wang Y-L, Hong A, Yen T-H, Hong H-H. Isolation of mesenchymal stem cells from human alveolar periosteum and effects of vitamin D on osteogenic activity of periosteum-derived cells. J Vis Exp. 2018;(135):57166. https://doi.org/10.3791/57166

32. Saedi AA, Debruin D, Hayes A, Kremer R, Duque G. Elucidating the genetic effect of vitamin D on mesenchymal stem cell differentiation in vitro. Europe PMC. 2022;1:1-12. https://doi.org/10.21203/rs.3.rs-1281522/v1

33. Posa F, Benedetto AD, Cavalcanti-Adam EA, Colaianni G, Porro C, Trotta T, et al. Vitamin D promotes MSC osteogenic differentiation stimulating cell adhesion and $\alpha V\beta 3$ expression. Stem Cells Int. 2018; 6958713. https://doi.org/10.1155/2018/6958713

34. Uberti F, Lattuada D, Morsanuto V, Nava U, Bolis G, Vacca G, et al. Vitamin D protects human endothelial cells from oxidative stress through the autophagic and survival pathways. J Clin Endocrinol Metab. 2014;99(4):1367–74. https://doi.org/10.1210/jc.2013-2103

35. Wang Y, Keulenaer GWD, Lee RT. Vitamin D_3-up-regulated protein-1 Is a stress-responsive gene that regulates cardiomyocyte viability through interaction with thioredoxin. J Biol Chem. 2002;277(29):26496-500. https://doi.org/10.1074/jbc.m202133200

36. Yanagisawa J, Yanagi Y, Masuhiro Y, Suzawa M, Watanabe M, Kashiwagi K, et al. Convergence of transforming growth factor-β and vitamin D signaling pathways on SMAD transcriptional coactivators. Science. 1999;283(5406):1317-21. https://doi.org/10.1126/science.283.5406.1317

37. Zhou J, Wang F, Ma Y, Wei F. Vitamin D_3 contributes to enhanced osteogenic differentiation of MSCs under oxidative stress condition via activating the endogenous antioxidant system. Osteoporos Int. 2018;29(8):1917–26. https://doi.org/10.1007/s00198-018-4547-0

38. Wang D, Lin L, Lei K, Zeng J, Luo J, Yin Y, et al. Vitamin D_3 analogue facilitates epithelial wound healing through promoting epithelial-mesenchymal transition via the Hippo pathway. J Dermatol Sci. 2020;100(2):120-128. https://doi.org/10.1016/j.jdermsci.2020.08.015

39. Rebsamen MC, Sun J, Norman AW, Liao JK. 1α,25-Dihydroxyvitamin D_3 induces vascular smooth muscle cell migration via activation of phosphatidylinositol 3-kinase. Circ Res. 2002;91(1):17-24. https://doi.org/10.1161/01.res.0000025269.60668.0f

40. Pittarella P, Squarzanti DF, Molinari C, Invernizzi M, Uberti F, Renò F. NO-dependent proliferation and migration induced by Vitamin D in HUVEC. J Steroid Biochem Mol Biol. 2015;149:35-42. https://doi.org/10.1016/j.jsbmb.2014.12.012

41. Sattary M, Rafienia M, Kazemi M, Salehi H, Mahmoudzadeh M. Promoting effect of nano hydroxyapatite and vitamin D_3 on the osteogenic differentiation of human adipose-derived stem cells in polycaprolactone/gelatin scaffold for bone tissue engineering. Mater Sci Eng C Mater Biol Appl. 2019;97:141-155. https://doi.org/10.1016/j.msec.2018.12.030

42. Merimi M, El-Majzoub R, Lagneaux L, Moussa Agha D, Bouhtit F, Meuleman N, et al. The therapeutic potential of mesenchymal stromal cells for regenerative medicine: current knowledge and future understandings. Front Cell Dev Biol. 2021;9:661532. https://doi.org/10.3389/fcell.2021.661532

43. Nam D, Park A, Dubon MJ, Yu J, Kim W, Son Y, et al. Coordinated regulation of mesenchymal stem cell migration by various chemotactic stimuli. Int J Mol Sci. 2020;21(22):8561. https://doi.org/10.3390/ijms21228561

44. Ryu CH, Park SA, Kim SM, Lim JY, Jeong CH, Jun JA, et al. Migration of human umbilical cord blood mesenchymal stem cells mediated by stromal cell-derived factor-1/CXCR4 axis via Akt, ERK, and p38 signal transduction pathways. Biochem Biophys Res Commun. 2010 Jul 16;398(1):105-10. https://doi.org/10.1016/j.bbrc.2010.06.043

45. Naaldijk Y, Johnson AA, Ishak S, Meisel HJ, Hohaus C, Stolzing A. Migrational changes of mesenchymal stem cells in response to cytokines, growth factors, hypoxia, and aging. Exp Cell Res. 2015;338(1):97-104. https://doi.org/10.1016/j.yexcr.2015.08.019

46. Coperchini F, Greco A, Croce L, Petrosino E, Grillini B, Magri F, et al. Vitamin D reduces thyroid cancer cells migration independently from the modulation of CCL2 and CXCL8 chemokines secretion. front endocrinol. 2022;13:876397. https://doi.org/10.3389/fendo.2022.876397

47. Ji J, Cheng X, Wang W, Zhang J. Vitamin D regulates cell viability, migration and proliferation by suppressing galectin-3 (Gal-3) gene in ovarian cancer cells. J Biosci. 2020;45(1):69. http://doi.org/10.1007/s12038-020-00038-1

48. Liu Z, Sun H, Chen Y, He J, Zhu L, Yang B, et al. High glucose-induced injury in human umbilical vein endothelial cells is alleviated by vitamin D supplementation through downregulation of TIPE1. Diabetol Metab Syndr. 2024;16(1):18. https://doi.org/10.1186/s13098-024-01264-5

49. Viveiros MMH, Viveiros MEDM, Silva MG, Kaneno R, Avelino NP, Rainho CA, et al. Expression of inflammatory cytokines in mesenchymal stem cells derived from proximal humerus fractures. Stem Cell Investig. 2022;9:3–3. https://doi.org/10.21037/sci-2021-031

50. Beldi G, Khosravi M, Abdelgawad ME, Salomon BL, Uzan G, Haouas H, et al. TNFα/TNFR2 signaling pathway: An active immune checkpoint for mesenchymal stem cell immunoregulatory function. Stem Cell Res Ther. 2020;11(1):281. https://doi.org/10.1186/s13287-020-01740-5

51. Egea V, von Baumgarten L, Schichor C, Berninger B, Popp T, Neth P, et al. TNF-α respecifies human mesenchymal stem cells to a neural fate and promotes migration toward experimental glioma. Cell Death Differ. 2011;18(5):853–63. https://doi.org/10.1038/cdd.2010.154

52. Cardus A, Panizo S, Encinas M, Dolcet X, Gallego C, Aldea M, et al. 1,25-Dihydroxyvitamin D_3 regulates VEGF production through a vitamin D response element in the VEGF promoter. Atherosclerosis. 2009;204(1):85–9. https://doi.org/10.1016/j.atherosclerosis.2008.08.020

53. Kagiwada H, Yashiki T, Ohshima A, Tadokoro M, Nagaya N, Ohgushi H. Human mesenchymal stem cells as a stable source of VEGF-producing cells. J Tissue Eng Regen Med. 2008;2(4):184–9. https://doi.org/10.1002/term.79

54. Fierro FA, Magner N, Beegle J, Dahlenburg H, Logan White J, Zhou P, et al. Mesenchymal stem/stromal cells genetically engineered to produce vascular endothelial growth factor for revascularization in wound healing and ischemic conditions. Transfusion. 2019;59(S1):893–7. https://doi.org/10.1111/trf.14914

55. Schmelzer E, McKeel DT, Gerlach JC. Characterization of human mesenchymal stem cells from different tissues and their membrane encasement for prospective transplantation therapies. Biomed Res Int. 2019;2019:6376271. https://doi.org/10.1155/2019/6376271

56. Shin S, Lee J, Kwon Y, Park K-S, Jeong J-H, Choi S-J, et al. Comparative proteomic analysis of the mesenchymal stem cells secretome from adipose, bone marrow, placenta and Wharton's jelly. IJMS. 2021;22(2):845. https://doi.org/10.3390/ijms22020845

57. Yang C-Y, Chang P-Y, Chen J-Y, Wu B-S, Yang A-H, Lee OK-S. Adipose-derived mesenchymal stem cells attenuate dialysis-induced peritoneal fibrosis by modulating macrophage polarization via interleukin-6. Stem Cell Res Ther. 2021;12(1):193. https://doi.org/10.1186/s13287-021-02270-4

58. Kwon DH, Hwang J, You H, Kim NY, Lee GY, Han SN. Effects of an in vitro Vitamin D Treatment on the Inflammatory Responses in Visceral Adipose Tissue from Ldlr−/− mice. Nutr Res Pract. 2024;18(1):19. https://doi.org/10.4162/nrp.2024.18.1.19

59. Deng W, Chen W, Zhang Z, Huang S, Kong W, Sun Y, et al. Mesenchymal stem cells promote CD206 expression and phagocytic activity of macrophages through IL-6 in systemic lupus erythematosus. Clin Immunol. 2015;161(2):209-16. https://doi.org/10.1016/j.clim.2015.07.011

60. He M, Shi X, Yang M, Yang T, Li T, Chen J. Mesenchymal stem cells-derived IL-6 activates AMPK/mTOR signaling to inhibit the proliferation of reactive astrocytes induced by hypoxic-ischemic brain damage. Exp Neurol. 2019;311:15-32. https://doi.org/10.1016/j.expneurol.2018.09.006

61. Saparov A, Ogay V, Nurgozhin T, Jumabay M, Chen WCW. Preconditioning of human mesenchymal stem cells to enhance their regulation of the immune response. Stem Cells Int. 2016;2016:3924858. https://doi.org/10.1155/2016/3924858

62. Noronha NC, Mizukami A, Caliári-Oliveira C, Cominal JG, Rocha JLM, Covas DT, et al. Priming approaches to improve the efficacy of mesenchymal stromal cell-based therapies. Stem Cell Res Ther. 2019;10(1):131. https://doi.org/10.1186/s13287-019-1259-0

63. Moeinabadi-Bidgoli K, Mazloomnejad R, Maal AB, Aghdaei HA, Arki MK, Hossein-Khannazer N, et al. Genetic modification and preconditioning strategies to enhance functionality of mesenchymal stromal cells: a clinical perspective. Expert Opin Biol Ther. 2023;23(6):461-478. https://doi.org/10.1080/14712598.2023.2205017

64. Miceli V, Zito G, Bulati M, Gallo A, Busà R, Iannolo G, et al. Different priming strategies improve distinct therapeutic capabilities of mesenchymal stromal/stem cells: Potential implications for their clinical use. World J Stem Cells. 2023;15(5):400–20. https://doi.org/10.4252/wjsc.v15.i5.400