

Effects of Hypoxia and Lipopolysaccharide on the Migration Capacity and Paracrine Factor Expression of Human Umbilical Cord Mesenchymal Stem Cells

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ABSTRACT

Background: Mesenchymal stem cells (MSCs) have emerged as a promising strategy for tissue regeneration. Although hypoxia or lipopolysaccharide (LPS) has been shown to affect the regenerative capacity of MSCs, their combined effects on MSCs are still unknown. We examined the effects of hypoxia and LPS on MSC migration rates and paracrine expression.

Materials and Methods: Umbilical cord-derived MSCs (UC-MSCs) were isolated and characterized. Wound scratch assays assessed migration and proliferation under hypoxic conditions, LPS supplementation, or both. The expression of paracrine factors was analyzed by quantitative real-time polymerase chain reaction (qRT-PCR).

Results: Twenty-four hours post-scratching, the gap closure in UC-MSCs after both hypoxia exposure and LPS treatment was significantly enhanced than those in the control group ($p=0.008$) or those subjected to individual treatments ($p=0.028$ vs. hypoxia and $p=0.0021$ vs. LPS). Serum-supplemented media further maximized these synergistic effects. Combined hypoxia and LPS did not affect vascular endothelial growth factor (VEGF) mRNA levels. In contrast, both hypoxia exposure and LPS treatment decreased the transcript levels of hepatocyte growth factor (HGF), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and transforming growth factor- β (TGF- β).

Conclusion: These findings indicate that the combination of hypoxia and LPS treatment affects the proliferative capacity of UC-MSCs, thus enhancing the MSC migration rate. However, hypoxia and LPS together reduce the expression of paracrine factors in UC-MSCs, potentially attenuating the immunomodulatory efficacy of UC-MSCs in tissue injury microenvironments.

Keywords: Hypoxia; Lipopolysaccharide; Mesenchymal stem cells; Migration; Proliferation

INTRODUCTION

Tissue injury and inflammation are hallmarks of numerous pathological conditions ranging from trauma and ischemic events to chronic inflammatory diseases [1]. In these microenvironments, the cells are exposed to a combination of hypoxia^{2,3}, reduced oxygen availability, and inflammatory signals such as

lipopolysaccharide (LPS)⁴. These factors exacerbate tissue damage and play pivotal roles in regulating cellular responses^{5,6}, including mesenchymal stem cells (MSCs), which are critical players in tissue regeneration and repair^{7,8}.

MSCs are a promising therapeutic option for many diseases, including cardiovascular disorders,

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neurodegenerative conditions, musculoskeletal injuries, and immune-mediated diseases^{9–11}. Their self-renewal, multipotency, and ability to migrate to injury sites, make them attractive for regenerative medicine. MSCs exert therapeutic effects primarily through the secretion of paracrine factors that modulate immune responses, promote tissue regeneration, and reduce inflammation. These paracrine factors include growth factors, cytokines, and exosomes, which create a pro-regenerative microenvironment and enhance repair and functional recovery in damaged tissues^{12,13}.

Hypoxia is a common consequence of tissue injury, particularly in ischemic conditions where the blood supply is compromised. Reduced oxygen levels impair cellular metabolism and function, triggering a cascade of events, including hypoxia-inducible factor-1 α (HIF-1 α) stabilization. HIF-1 α orchestrates adaptive responses, such as angiogenesis, glycolytic metabolism, and cell survival pathways, to promote tissue recovery^{14,15}. Under hypoxic conditions, MSCs exhibit an enhanced migratory capacity, allowing them to home to injury sites more effectively. Additionally, hypoxia stimulates MSC proliferation^{16,17}, potentially increasing their regenerative capacity. Most significantly, hypoxia alters the secretory profile of MSCs, promoting the release of paracrine factors that play a crucial role in modulating the local tissue environment, promoting angiogenesis, reducing inflammation, and supporting tissue repair¹⁸.

LPS-mediated inflammation is often observed when tissue injury is accompanied by infection or bacterial translocation^{19,20}. LPS, a potent endotoxin, binds to toll-like receptor 4 (TLR4) on cell surfaces, initiating an inflammatory cascade characterized by the release of proinflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and interleukin-1 beta (IL-1 β)²¹. Although inflammation is a necessary component of the initial immune response to injury, excessive or prolonged inflammatory signaling can impair tissue regeneration and exacerbate cellular dysfunction²². MSCs respond dynamically to LPS stimulation, altering their paracrine activity, migratory behavior, and proliferative capacity to mediate immune modulation and tissue repair^{23,24}.

The dual presence of hypoxia and LPS in damaged tissues creates a unique and challenging microenvironment for MSCs. Understanding how these factors influence MSC migration, proliferation, and paracrine signaling is essential for elucidating the mechanisms underlying tissue repair and optimizing the therapeutic potential of MSC-based therapies. Although previous studies have explored the individual effects of hypoxia and LPS on MSCs^{23,25}, few studies have characterized their combined influence on umbilical cord-derived MSCs (UC-MSCs) functional capacity. By elucidating how MSCs respond to these stimuli, this study provides insights into novel strategies to enhance MSC-based regenerative therapies for the treatment of damaged and inflamed tissues.

MATERIALS AND METHODS

Isolation and Expansion of Umbilical Cord-MSCs

UC-MSCs were isolated from the Wharton's jelly (WJ) samples collected from mothers aged 20–35 years after cesarean delivery and cultured using the explant method²⁶ with a few modifications as previously described²⁵. Briefly, the UC was transversely incised to a length of 5 cm. WJ was separated from the UC, both umbilical arteries and veins were removed, and the WJ was cut into small pieces and 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), 1% Glutamax, and 1% penicillin–streptomycin. The MSCs were cultured in a humidified incubator at 37°C with 5% CO₂. The culture medium was replaced every 2–3 days, and the cells were passaged when they reached 80–90% confluence using 0.25% trypsin-EDTA (Sigma-Aldrich, Darmstadt, Germany). The UC-MSCs were characterized as previously described²⁵. The UC-MSCs used in these experiments were at passages 3–5.

Pre-conditioning of MSCs with Hypoxia Exposure and Lipopolysaccharide Treatment

Hypoxic conditions were established using cobalt (II) chloride hexahydrate (CoCl₂, Sigma-Aldrich, Darmstadt, Germany). Upon reaching approximately

80% confluence, the culture medium of MSCs was replaced with fresh medium supplemented with 100 μ M CoCl₂. For LPS exposure, the culture medium was supplemented with 10 ng/mL LPS (Sigma-Aldrich, St. Louis, MO, USA), followed by a 24 h incubation period. Stock solutions of 25 mM CoCl₂ and 100 ng/mL LPS were prepared in sterile distilled water. The CoCl₂ dosage was determined based on the study by Arfianti et al²⁵.

Scratch Wound Healing Assay

Scratch wound healing assay was performed to measure the migration capacity of UC-MSCs cultured under hypoxic conditions, LPS supplementation, or a combination of both. MSCs were cultured in six-well plates at a density of $5 \times 10^4/\text{cm}^2$. After cell confluency reached 70–80%, the scratched cell-free zone was manually created across the cell monolayer using a sterile 1000 μ L pipette tip. The cells were then washed twice with Dulbecco's phosphate-buffered saline (DPBS) (Sigma-Aldrich, Taufkirchen, Germany) to remove cellular debris. Subsequently, culture medium containing CoCl₂ and/or LPS was added, and the cells were cultured for 24 h. The migration of UC-MSCs was observed and photographed using an inverted microscope (Olympus CKX53). The wound area was measured using the ImageJ software. Wound closure percentage was calculated using the following formula: wound closure rate (%) = $(T_0 - T_t)/T_0 \times 100$, where T_0 is the wound area at 0 h, and T_t is the remaining area at the designated time²⁷.

Quantitative Real-time Polymerase Chain Reaction

In these experiments, MSCs at passages 3–5 were used once 80–90% confluency were achieved. After the cells were pre-conditioned via hypoxia exposure and/or LPS treatment for 24 h, the conditioned medium was aspirated, and total RNA was extracted using TRNzol Universal Reagent (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. The concentration of extracted RNA was measured using a Multiskan SkyHigh Microplate Spectrophotometer (Thermo Fisher Scientific). Using 1 μ g of total RNA, the GoTaq® 2-Step qRT-PCR system (Promega Co., Madison, WI, USA) was used for cDNA synthesis. The targeted genes were amplified using

specific primers (Table 1)^{25,26,28} 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). qRT-PCR was performed using GoTaq® 2-Step qRT-PCR (Promega Co., Madison, Wisconsin, USA). Comparative Ct ($2^{-\Delta\Delta C_t}$) was used to compare the expression of the target genes normalized to that of GAPDH as a reference control. Melting curve analysis was performed to determine the specificity of the PCR products.

Data analysis

Data are presented as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) was used to test the differences on cell migration, proliferation, and secretion of paracrine factors between experimental groups. Post-hoc analysis was performed using Tukey's multiple comparison test. Statistical analysis was performed using GraphPad Prism v 9 and a $p < 0.05$ was deemed statistically significant.

RESULT

MSCs were isolated from WJ of the human UC, expanded, and characterized based on cell surface markers using flow cytometry²⁵. As shown in Figure 1, 99.9 and 99.8% the UC-MSCs population displayed positive cell surface markers CD90 and CD73 in of the cells, respectively. As for another specific marker, CD105 was only expressed on 62.1% of MSCs.

Effect of Hypoxia Exposure and LPS Treatment on UC-MSC Migration Capacity

To measure the ability of UC-MSCs to migrate exclusively without being influenced by their proliferation capacity, a wound scratch assay was performed on UC-MSCs cultured in serum-free media. Figure 2A shows the gap area immediately (0 h) and 24 h after scratching (24 h). The percentage of gap closure in all groups after 24 h of scratching is shown in Fig. 2B. UC-MSCs cultured in a combination of LPS and hypoxic conditions showed 64.9% gap closure compared to 45.4% in the control group. UC-MSC treated with LPS showed a higher rate (84.2%), whereas those cultured under hypoxia demonstrated 52% gap closure. Despite these differences, the percentage of gap closure was not significantly

different between the treatment groups ($p>0.05$).

Table 1. RT-qPCR Primer Sequences

Gene	Forward	Reverse
GAPDH	GACCTGCCGTCTAGAAAAAC	TTGAAGTCAGAGGAGACCAC
HGF	GGTAAAGGACGCAGCTACAAG	ATAACTCTCCCCATTGCAGGT
TGF- β	GCAGAAAGTTGGCATGGTAG	CCCTGGACACCAACTATTGC
TNF- α	TGGCCAATGGCGTGGAGCTG	GTAGGAGACGGCGATGCGGC
VEGF	ATCTGCATGGTGATGTTGGA	GGGCAGAATCATCAC GAAG
IL-6	AAGCCAGAGCTGTGCAGATGAGTA	TGTCCTGCAGCCACTGGTTC

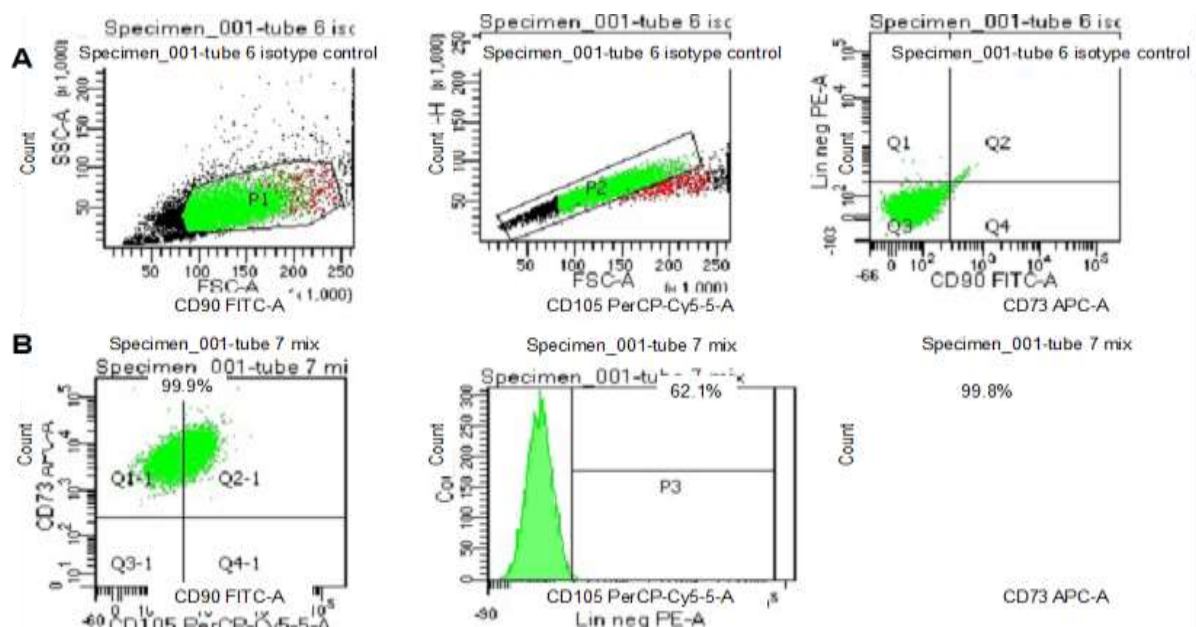


Figure 1. Immunophenotyping characterization of umbilical cord mesenchymal stem cells (UC-MSCs). Cells at passage 3 were labelled with monoclonal antibodies for (A) isotype control, (B) CD90, C105, CD73, and analyzed by flow cytometry

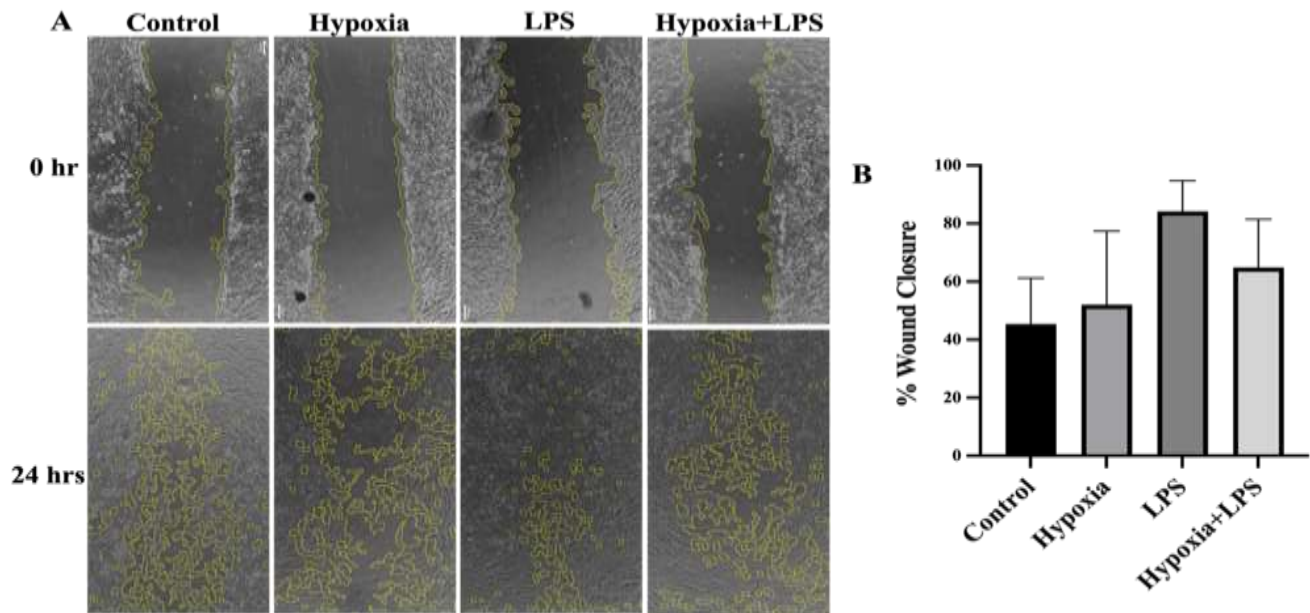


Figure 2. Effect of hypoxia exposure and LPS treatment on UC-MSC migration rates in serum-free media. Cells at passage 3-5 were seeded on 24-well culture plates with culture media containing 5% HPL until they reached 80% confluence. The wound scratch assay was created using a sterile 1000 μ L tip, and culture media were immediately replaced with serum-free culture media. The experimental group consisted of cells cultured under hypoxia conditions induced by 100 μ M CoCl_2 or exposed to 10 ng/mL LPS for 24 h or combined hypoxia and LPS treatment ($n=3$). (A) Images were captured immediately at 0 h and 24 h after scratching (original magnification 4 $\times$). (B) The percentage scratch closure was measured by ImageJ. Data represent mean $\pm$ SD. CoCl_2 , cobalt (II) chloride; HPL, human platelet lysate; LPS, lipopolysaccharide; UC-MSC, umbilical cord mesenchymal stem cell

Effect of Hypoxia Exposure and LPS Treatment on UC-MSC Proliferation Rate

To assess whether the migration capacity of UC-MSCs was influenced by their proliferative ability, a wound scratch assay was conducted in the presence of serum. Figure 3A shows the gap conditions immediately after scratching (0 h) and 24 h after scratching (24 h). Gap closure in UC-MSCs after 24 h of scratching is shown in Figure 3B. The percentage of gap closure in UC-MSCs cultured under hypoxic and normoxic conditions did not differ significantly (33.6% vs. 24.9%, $p>0.05$). UC-MSCs exposed to LPS exhibited lower gap closure than those in the control group (14.5% vs. 24.9%), although this difference was not statistically significant ($p>0.05$). However, the percentage of gap closure substantially increase in UC-MSCs cultured with a combination of LPS treatment and hypoxia exposure (67.1%), which was significantly different from those in the control ($p=0.008$), hypoxia ($p=0.028$), and LPS ($p=0.0021$) groups (Figure 3B). This indicated that the combination of hypoxia and LPS treatment increased

UC-MSC proliferation rate, leading to enhanced migration capacity.

Effect of Hypoxia Exposure and LPS Treatment on the Expression of UC-MSC Paracrine Factors

The effects of hypoxic culture and LPS treatment on the expression of paracrine factors in UC-MSCs, including growth factors and cytokines, were evaluated by qRT-PCR. As illustrated in Figure 4, the *VEGF* mRNA levels remained statistically comparable across all groups (Figure 4A). While neither hypoxia nor LPS affected the level of *HGF* mRNA, combined treatment decreased it compared to that in the control group ($p=0.0246$, Figure 4B). Furthermore, the level of *IL-6* mRNA was significantly lower in UC-MSCs cultured under hypoxic conditions ($p=0.0021$) and those received combined treatments ($p=0.0019$) than in the control group (Fig. 4C). Similarly, the levels of *TNF- α* and *TGF- β* mRNA were markedly decreased in hypoxic and LPS-treated UC-MSC compared to those

in control UC-MSCs ($p=0.0071$ and $p=0.0126$, respectively, Figure 4D-E).

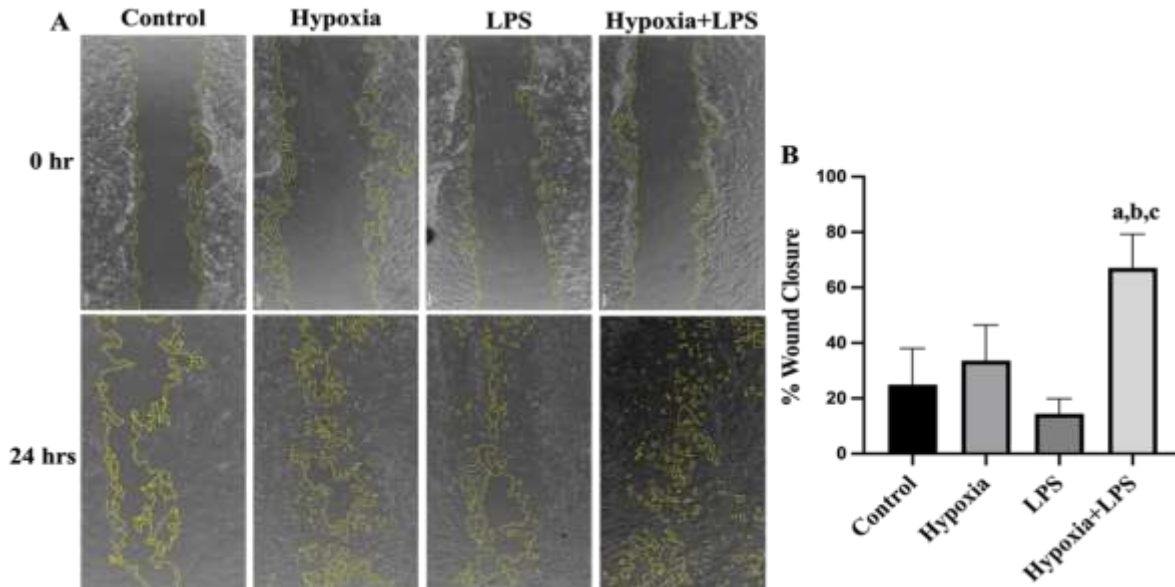


Figure 3. Effect of hypoxia exposure and LPS treatment on UC-MSC migration rates in serum-supplemented media. Cells at passage 3-5 were seeded on 24-well culture plates with culture media containing 5% HPL until they reached 80% confluence. The wound scratch assay was created using a sterile 1000 μ L tip, and cells were cultured with serum-containing media. The experimental group consisted of cells cultured under hypoxia condition induced by 100 μ M COCl_2 or exposed to 10 ng/mL LPS for 24 h or combined hypoxia and LPS treatment ($n=3$). (A) Images were captured immediately at 0 h and 24 h after scratching (original magnification 4 $\times$). (B) The percentage scratch closure was measured by ImageJ. Data represent mean $\pm$ SD. ^a $p=0.008$ vs. control; ^b $p=0.028$ vs. hypoxia; ^c $p=0.0021$ vs. LPS group. CoCl_2 , cobalt (II) chloride; HPL, human platelet lysate; LPS, lipopolysaccharide; UC-MSC, umbilical cord mesenchymal stem cell.

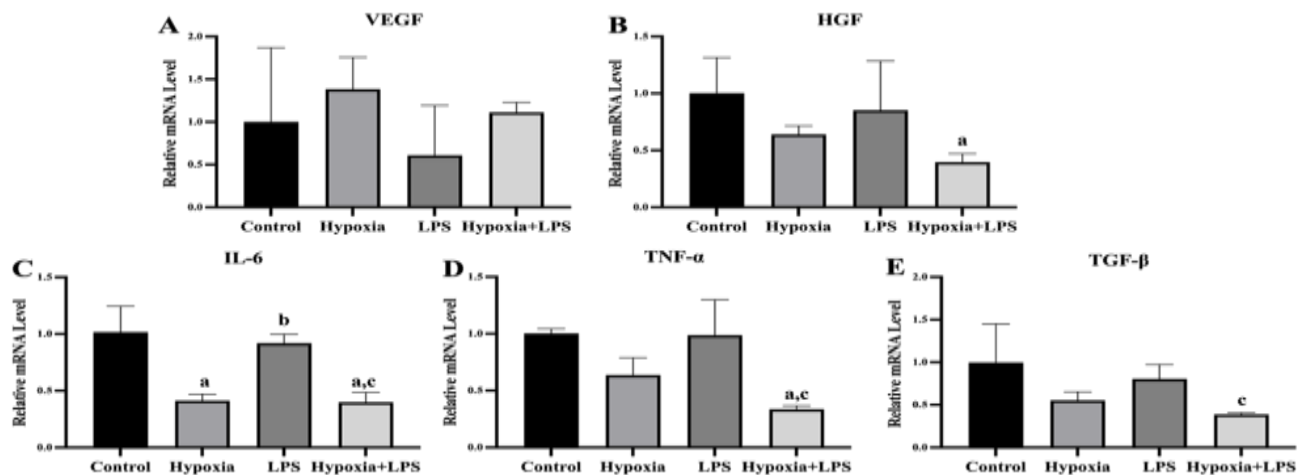


Figure 4. Effects of hypoxia and LPS on the gene expression in UC-MSC. Cells were cultured in hypoxia condition induced by COCl_2 100 μ M and/or exposed with 10 ng/mL LPS in culture media for 24 hrs ($n=3$). The mRNA levels of (A) VEGF, (B) HGF, (C) IL-6, (D) TNF- α , and (E) TGF- β were measured by RT-qPCR. Data were presented as mean $\pm$ SD. ^a $p<0.05$ vs. control; ^b $p<0.05$ vs. hypoxia; ^c $p<0.05$ vs. LPS group. UC-MSC, umbilical cord mesenchymal stem cell; HGF, hepatocyte growth factor; IL-6, interleukin-6; LPS, lipopolysaccharide; TNF- α , tumor necrosis factor- α ; TGF- β , transforming growth factor- β ; VEGF, vascular endothelial growth factor.

DISCUSSION

Inflammation is a fundamental driver of tissue repair, coordinating the cellular events necessary for successful wound healing. Hypoxia, which is frequently present in tissue injury, further influences the inflammatory microenvironment and can modulate the behavior of immune cells and other key players in the regeneration process [28]. MSCs have been extensively studied for tissue regeneration owing to their ability to modulate immune responses, promote regeneration, and maintain tissue homeostasis [13]. As this field of study advances, a clear understanding of the complex interactions among MSCs, inflammation, and hypoxia will be crucial for developing more effective regenerative therapies and optimizing MSC-based therapy outcomes.

The migration capacity of MSCs plays a critical role in their therapeutic potential, as it directly affects their ability to reach and repair damaged tissues. Effective migration allows MSCs to home to injury sites where they can exert their regenerative effects, thus enhancing overall treatment outcomes^{29,30}. This study revealed that the combination of hypoxia and LPS treatment significantly enhanced the migration rate of UC-MSCs, particularly in serum-containing culture media. This finding suggests that the proliferative capacity of UC-MSCs is a key factor in enhancing the migratory response under these conditions. The synergistic effect of hypoxia and LPS on MSC migration has not been extensively studied, although previous research has focused on the individual effects of these conditions. Few studies have investigated the interaction between hypoxia and other inflammatory mediators, including interferon- γ (INF- γ), TNF- α , and IL-6^{31,32}. While our findings demonstrated a positive impact of combined hypoxia exposure and LPS treatment on UC-MSC migration, the existing literature presents conflicting results. Consistent with our observations, TNF- α and INF- γ significantly enhanced the migratory capacity of rat UC-MSCs under hypoxic conditions³². While hypoxia and inflammatory stimuli independently enhanced bone marrow-derived MSC (BM-MSC) migration in both *in vitro* and *in vivo* settings, their combination did not confer a synergistic effect³³. In contrast, other studies have shown that pre-

conditioning with hypoxia exposure or inflammatory mediator treatment did not significantly affect the migration of BM-MSCs. Interestingly, the expression of migration and adhesion receptors were altered when inflammatory factors (INF- γ /TNF- α) were added to the culture medium, although this did not translate into changes in MSC migration *in vitro* or adhesion *in vivo*³⁴. These contrasting findings highlight the complexity of MSC migration mechanisms and underscore the need for further research to elucidate the precise factors and conditions that optimize MSC migration for therapeutic applications.

While this study showed that hypoxia alone did not influence the migration capacity of UC-MSC, opposing data have been reported in other studies. For instance, hypoxic pre-conditioning significantly increased the migratory ability of placental-derived MSCs, which was attributed HIF-1 α upregulation and miR-656-3p downregulation of³⁵. Similarly, hypoxia not only promoted the migration capacity of dental pulp-MSCs, but also enhances their survival and proliferation³⁵. Comparable positive effects of hypoxia on MSC migration have been observed in other types of MSCs, including those derived from the human amniotic fluid³⁶ and dental pulp^{35,37}. The discrepancies observed in these studies could be attributed to differences in cell sources, oxygen concentrations, and the methodologies used for assessing the migration rates of MSCs.

Moreover, our findings indicated that the presence of serum in the culture media is crucial for the enhanced migration rates of UC-MSCs in response to the combination of hypoxia and LPS. *In vitro* studies demonstrated that serum plays a crucial role in enhancing the migratory capacity of MSCs. BM-derived MSCs cultured with fetal bovine serum (FBS) exhibited increased migration compared to MSCs cultured in serum-free conditions³⁸. Similarly, adipose tissue-derived stem cells (ADSCs) exposed to sepsis serum showed enhanced migratory capabilities, suggesting that specific serum conditions, even those mimicking pathological states, can augment the MSC migration potential³⁹. Human blood serum significantly increases the migration rates and distance of cardiac stem cells, underscoring its positive effect on stem cell

migration⁴⁰. Furthermore, serum rich in growth factors, such as those derived from human platelets, enhances the migration capacity of ADSCs compared with standard FBS [41]. The enhancement of migration by serum is often mediated through specific signaling pathways, such as the p38 mitogen-activated protein kinase (MAPK pathway), which is activated in MSCs in the presence of human blood serum [40]. Serum conditions that promote migration have been associated with downregulation of certain proteins involved in the migration machinery, such as α -smooth muscle actin (α SMA)⁴¹. Whether this *in vitro* enhanced migration capacity of MSCs will affect their therapeutic efficacy remains a subject for future research regarding the implementation of clinical applications.

Previous studies have shown that the secretion of trophic factors and cytokines is one of the mechanisms underlying the therapeutic effects of MSCs. This study revealed that combined treatment of hypoxia and LPS significantly reduced HGF gene expression in UC-MSCs. In contrast, LPS but not hypoxia was known to enhance HGF production on BM-MSCs⁴², although the synergistic effects of hypoxia and LPS on the secretion of HGF were not investigated. HGF, a pleiotropic cytokine secreted by MSCs, has multifaceted biological functions. In Alzheimer's disease models, HGF derived from MSCs demonstrates neuroprotective properties, including *tau* hyperphosphorylation attenuation, dendritic spine density restoration, and synaptic plasticity enhancement⁴³. HGF also acts as a potent osteogenic factor, promoting the differentiation of MSCs into osteoblasts, which is essential for osteogenesis and skeletal homeostasis⁴⁴. Furthermore, HGF exhibits immunomodulatory effects, as evidenced by its capacity to suppress antigen-presenting cell maturation and mitigate inflammatory responses, thereby enhancing graft survival in corneal transplantations⁴⁵. The regenerative potential of HGF is mediated through activation of the mesenchymal-epithelial transition factor (MET) receptor tyrosine kinase, which is expressed in various cell types⁴⁶. Owing to the important roles of HGF in the therapeutic efficacy of MSC, it is important to evaluate whether the findings of this study, which showed decreased HGF levels due to hypoxia and LPS

treatment, could affect the regenerative capacity of MSCs.

Similarly, our study showed that hypoxia and LPS treatment reduced TGF- β expression in UC-MSCs. In contrast to our findings, a previous study demonstrated that hypoxia increased TGF- β expression in BM-MSCs⁴⁷, while another study indicated that LPS treatment decreased TGF- β expression in induced pluripotent stem cell (iPSC)-derived MSCs⁴⁸. Hypoxia also enhanced the immunosuppressive capacity of BM-MSCs in hypoxic condition as shown by the inhibition of CD4⁺ T cell proliferation⁴⁹. Furthermore, this study demonstrated that the immunosuppressive function of BM-MSCs were mediated by TGF- β secretion, which led to the activation of autophagy process in CD4⁺ T cells. These conflicting results suggest that the effects of hypoxia and LPS on TGF- β expression may vary depending on the source of MSCs. Further research is needed to elucidate the specific mechanisms underlying these differences between UC-MSCs and BM-MSCs. Additionally, investigating the impact of hypoxia and LPS on other immunomodulatory factors produced by MSCs could provide a more comprehensive understanding of their effects on the cells' immunosuppressive properties.

Results describing the effects of hypoxia and LPS-treated MSCs on the expression of genes that encode inflammatory cytokines such as IL-8 and TNF- α are inconsistent. For instance, treatment of iPSC-MSCs with LPS highly increased the production of IL-8 and TNF- α ⁴⁸. Our study found that the combination of hypoxia exposure and LPS treatment downregulated of *IL-6* gene expression UC-MSCs. This effect might be part of an adaptive UC-MSC mechanism to cope with stress and to prevent excessive inflammation. The low expression of IL-6 and TNF- α in UC-MSCs in the presence of hypoxia and LPS could indicate that the influence of environmental factors on gene expression in MSCs seems to be complex, cell type-specific, and may be affected by additional factors and by interaction between signaling pathways.

Although this study offers useful insight into how hypoxia and LPS jointly affect UC-MSC migration, several limitations warrant discussion. First, we did not directly measure HIF-1 α protein expression, the

standard marker of cellular hypoxia. Low-oxygen conditions stabilized HIF-1 α , raising its protein levels⁵⁰, this is typically confirmed by Western blot^{51,52}. We instead used CoCl₂, a hypoxia-mimetic agent known to stabilize HIF-1 α ^{53,54}. Without direct measurement of HIF-1 α protein, however, we cannot be certain that hypoxic conditions were successfully induced in our experiments, and this limits how our findings should be interpreted. Future work should confirm these results and test whether other methods of inducing hypoxia produce similar effects on MSC migration and function.

A second limitation is that we assessed paracrine factor expression only at the mRNA level. While this captures the transcriptional response to hypoxia and LPS, mRNA changes do not always translate into corresponding protein changes because of post-transcriptional and post-translational regulation. Our results should therefore be read as evidence of transcriptional change rather than confirmed shifts in cytokine production. Protein-based methods such as enzyme-linked immunosorbent assay (ELISA) or proteomic analysis are needed to determine whether these gene expression changes correspond to functional alterations in the UC-MSC secretome.

Finally, although our data point to a synergistic effect of hypoxia and LPS on MSC migration, the broader literature is not entirely consistent. Our results agree with prior reports of enhanced UC-MSC migratory capacity under combined hypoxic and inflammatory conditions^{31,32}, but other studies, particularly those using BM-MSCs, have reported the opposite³⁴.

Such discrepancies likely reflect differences in experimental protocols, cell source, and migration assay. More broadly, MSC migration is assessed inconsistently across studies, and responses appear to vary by cell source. Future studies should adopt standardized migration assays and include HIF-1 α expression analysis to confirm hypoxic conditions, which would help refine the model and validate these findings.

CONCLUSION

Our study demonstrates that combined hypoxia exposure and LPS treatment enhance the gap closure capacity of UC-MSCs, most likely through increased cellular proliferation. In contrast, this enhanced

proliferative response was accompanied by reduced mRNA expression of several paracrine factors, including HGF, IL-6, TNF- α , and TGF- β , indicating that combined hypoxic and inflammatory stimulation differentially regulates the early biological responses of UC-MSCs.

Our findings contribute to a better understanding of how UC-MSCs respond to a microenvironment that mimics tissue injury and inflammation. Future studies should validate these findings by assessing HIF-1 α protein expression, investigating the signaling pathways involved, and determining whether the observed changes in gene expression are reflected at the protein level and persist over time. Such studies will help optimize MSC priming strategies to enhance their therapeutic potential in regenerative medicine.

Abbreviations

CCK-8 Assay: cell counting kit-8 assay
 CD: Cluster of differentiation
 DMEM: Dulbecco's modified Eagle's medium
 DPBS: Dulbecco's phosphate-buffered saline
 EDTA: ethylenediaminetetraacetic acid
 GAPDH: glyceraldehyde-3-phosphate dehydrogenase
 HGF: Hepatocyte growth factor
 HIF1- α : hypoxia-inducible factor 1-alpha
 HPL: human platelet lysate
 IL-1 β : interleukin-1 beta
 IL-6: interleukin-6; INF- γ , interferon- γ
 iPSC: induced pluripotent stem cell
 LPS: lipopolysaccharide
 MAPK: mitogen-activated protein kinase
 MET: mesenchymal-epithelial transition factor
 MSC: mesenchymal stem cell
 PBS: phosphate-buffered saline
 PI3K: phosphoinositide 3-kinase
 QRT-qPCR: quantitative real-time polymerase chain reaction
 TGF- β : transforming growth factor- β
 TLR4: toll-like receptor 4
 TNF- α : tumor necrosis factor- α
 SD: standard deviation
 UC: umbilical cord
 VEGF: vascular endothelial growth factor
 WJ: Wharton's jelly.

Availability of Data and Materials

All data reported in this paper will also be shared by the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

All the experimental procedures in this study were conducted in accordance with the 1964 Helsinki Declaration and its later amendments and approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia (Ethical code No: B/047/UN19.5.1.1.8/UEPKK/2021_Adendum3).

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Declaration of AI and AI-assisted Technologies in the Writing Process

The authors acknowledge the use of AI-assisted tools, including Consensus (<https://consensus.app/>) for literature search and Paperpal (<https://paperpal.com/>) for language refinement and manuscript editing. These tools were utilized to improve clarity and coherence but did not contribute to the conceptualization, data analysis, or scientific conclusions of this study. The authors take full responsibility for the content and integrity of the manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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