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Hypoxia-Induced Mesenchymal Stem Cell-Derived Exosome Enhance TGF-β, PDGF, and Hair Growth in Alopecia-Like Rat Models

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Abstract. Alopecia disrupts hair growth, leading to hair loss and baldness. Hypoxia-induced mesenchymal stem cell (MSC)–derived exosomes have emerged as a promising therapeutic alternative. These exosomes can modulate inflammatory responses and potentially enhance the expression of TGF-β and PDGF, key growth factors involved in hair follicle regeneration. This study aims to investigate the effects of hypoxia-induced MSC exosomes on TGF-β and PDGF expression in a fluconazole-induced alopecia-like rat model. Five groups were used: K1 (healthy control), K2 (fluconazole-induced, 0.9% NaCl), K3 (Minoxidil), K4 (100 µL hypoxia-induced MSC exosomes), and K5 (200 µL hypoxia-induced MSC exosomes). Skin samples were collected on day 15 post-treatment for analysis of TGF-β and PDGF levels using enzyme-linked immunosorbent assay (ELISA). Based on the analysis, the highest levels of TGF-β and PDGF were found in K1, followed by K5 and K4, while the lowest levels were observed in K2. This study finds that the single-dose injection of 200 µl of EH-MSCs daily for 14 days significantly increases the levels of TGF-β and PDGF in the skin, which contributes to improved hair growth. This study assumes that TGF-β and PDGF potentially improve hair growth through immunomodulation and a pro-angiogenic mechanism. These findings suggest that hypoxia-induced MSC exosomes represent a promising therapeutic strategy for alopecia and may be developed broadly for regenerative medicine targeting chronic inflammation-based skin disorders. However, further studies are needed to explore more about various cytokine and their function that relevance for alopecia and other inflammation-related skin conditions.

Keywords: alopecia; exosomes; hair follicle; hypoxia; MSC

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INTRODUCTION

Alopecia is a clinical manifestation of disrupted hair follicle growth cycles, primarily affecting the telogen phase, and is characterized by sudden hair shedding that can lead to baldness (Anindhita et al., 2018; Miao et al., 2022; Sibbald, 2023). Beyond its physiological manifestations, alopecia has profound psychosocial impacts, especially on women. Hair is closely tied to identity, femininity, and confidence. Consequently, women experiencing hair loss often suffer from emotional distress, diminished self-esteem, social withdrawal, and overall reduced quality of life, more severely than men with similar conditions (Ito et al., 2022).

Various treatment approaches, including conventional therapies such as topical corticosteroids, immunomodulators, and minoxidil, are commonly prescribed to stimulate hair regrowth (Lintzeri et al., 2022). However, their effectiveness is frequently limited, and adverse effects such as skin irritation, hypertrichosis, and hormonal imbalances are not uncommon (Dikeoulia et al., 2021; Nestor et al., 2021). On the other hand, surgical hair transplantation has gained popularity recently because of its natural appearance and more permanent results. However, this procedure is performed in an invasive approach, is expensive, and has complications in particular patients (Gardenia Akhyar & Nagara, 2024; Rambwawasvika, 2021). These several limitations underscore the need for more effective and safer

therapeutic strategies against alopecia.

Recent studies have reported regenerative medicine as a promising alternative, offering biologically targeted solutions that address the fundamental mechanisms underlying alopecia. Mesenchymal stem cell-derived exosome (E-MSCs), which are rich in growth factors including chemokines, cytokines, and microRNAs, performs promising results in hair follicle regeneration (Kim et al., 2020; Rahardja et al., 2024; Salhab et al., 2022). The growth factor components, such as transforming growth factor beta (TGF-β) (Mohamad et al., 2023) and platelet-derived growth factor (PDGF) (Jessika et al., 2024), have been proven to modulate signaling pathways in hair growth. TGF-β controls inflammation by activating regulatory T (Treg) cells that produce IL-10 and suppress inflammation. Meanwhile, PDGF is essential for promoting angiogenesis and supporting dermal papilla cell proliferation (Zhang et al., 2021), which is involved in maintaining hair follicle health (Pazzaglia et al., 2019). Another study also shows that decreased PDGF levels are directly linked to impaired vascularization that promotes alopecia progression (Mohanan et al., 2020).

The growth factor content in E-MSCs can be significantly increased under hypoxic conditions (1–5% O_2), thereby increasing their potential as therapeutic agents (Edward et al., 2022). This enhancement is primarily driven by the activation of hypoxia-inducible factor-1 alpha (HIF-1α), which also induces the transcription of TGF-β and upregulates PDGF gene expression (Fallah et al., 2022; Gómez-Ferrer et al., 2021). As a result, hypoxia-preconditioned MSCs demonstrate enhanced pro-angiogenic and regenerative capacities, particularly relevant to the restoration of damaged tissues such as hair follicles (Yang et al., 2022). Despite increasing scientific interest in the therapeutic potential of E-MSCs, studies specifically exploring the role of exosome hypoxia MSCs in modulating TGF-β and PDGF signaling in the context of alopecia remain very limited. Therefore, this study aims to evaluate the therapeutic potential of exosome hypoxia MSCs by analyzing their effect on TGF-β and PDGF expression in a fluconazole-induced rat model of alopecia. This research aims to provide not only a novel therapeutic approach but also new mechanistic insights that may inform future regenerative interventions. The findings are expected to make a meaningful contribution to the development of safe, non-invasive, and immunomodulatory treatments for hair loss disorders.

METHODS

Study Design and Animal Model

This study employed a post-test-only control group design using a total of 25 8-week-old female Wistar rats weighing between 200 and 250 grams, which were obtained from the Stem Cell and Cancer Research (SCCR) Laboratory in Semarang City, Central Java, Indonesia. All experimental procedures involving animals were approved by the Ethics Committee of the Faculty of Medicine, Universitas Islam Sultan Agung, Semarang City, Ethical Clearance No: 3/I/2025/Komisi Bioetik. All rats were acclimated at 60% humidity, with temperatures ranging from 20°C to 24°C, a 12– to 14-hour light-dark cycle, continuous ventilation, and were provided with standard feed and water ad libitum throughout the study period.

Alopecia-like Model Induction

The rats were randomly divided into five groups (n = 5 per group), consisting of one healthy control group and four groups subjected to alopecia-like conditions. Fluconazole, administered orally at a dose of 15 mg/kg/day for seven consecutive days, was used to simulate antifungal-induced hair loss in alopecia-like conditions (Thompson et al., 2019).

Mesenchymal Stem Cell (MSC) Isolation and Characterization

Mesenchymal stem cells (MSCs) were isolated aseptically from umbilical cord tissue obtained from fetal rats, following the protocol by Nugraha & Putra (2018). The umbilical cords were washed with 0.9% NaCl and phosphate-buffered saline (PBS) to remove blood vessels. The tissue was finely chopped and then placed into sterile 25T flasks and left for 3 minutes to ensure proper adhesion. Then, 5 mL of media containing Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS), 0.25% fungizone, and 1% penicillin-streptomycin was poured into the flasks until the tissue layer was covered. Cells were incubated at 37°C with 5% CO_2 and monitored for up to 14 days. The media were partially replaced every 3 days until the cultures reached 80% confluence, at which point they were passed.

The MSCs' culture appearance was then confirmed by spindle-shaped morphology, followed by flow cytometric analysis of specific surface markers CD90 and CD29, which yielded

positive results (Akpinar et al., 2021), and CD45 and CD31, which yielded negative results (Shi et al., 2023). Cellular differentiation capacity was validated by culturing cells in osteogenic and adipogenic media, followed by Alizarin Red S (Sigma-Aldrich: Hamburg, Germany) and Oil Red O staining (Sigma-Aldrich: Hamburg, Germany), respectively. The MSC cultures were maintained until they reached 80% confluency before being exposed to hypoxic conditions.

Hypoxic Conditioning and Secretome Collection

The hypoxic condition was performed using a modified protocol adapted from Fredianto et al. (2023). In this study, the cultured MSC cells were incubated at 37°C for 24 hours in serum-free DMEM medium inside a hypoxia chamber flushed with nitrogen gas until the oxygen concentration reached 5%. After that, the conditioned medium was processed for exosome-containing secretome collection using tangential flow filtration (TFF) with a 100–300 kDa membrane.

Exosome Isolation, Characterization, and Treatment

Exosomes-contained secretomes isolated from the conditioned medium were cultured under serum-free conditions for 24 hours to prevent contamination. The isolated exosomes were validated using flow cytometry targeting exosomal surface markers CD81, CD63, and CD9. These three markers are tetraspanins commonly used to identify exosomes, as recommended by the International Society for Extracellular Vesicles (ISEV) (Fan et al., 2023; Mathieu et al., 2021). Hypoxia-induced MSC-derived exosome treatment was administered as a single injection on day 1 after confirmation of the alopecia-like condition and then continued for 14 days, as shown in Table 1.

PDGF and TGF-β Analysis using ELISA

Skin tissue samples were collected on day 15 post-injection from the alopecia treatment sites of each animal group. The samples were immediately rinsed with sterile phosphate-buffered saline (PBS) (Sigma-Aldrich, Hamburg, Germany) to eliminate surface contaminants and subsequently homogenized in cold PBS (pH 7.4) using a tissue homogenizer under sterile conditions. The homogenate was then treated with RIPA buffer supplemented with a protease inhibitor to prevent protein degradation. The homogenized samples were centrifuged, and the resulting supernatant was collected for quantification of TGF-β and PDGF levels. The homogenates were then centrifuged at 10,000 rpm for 10 minutes at 4 °C to remove cellular debris. The resulting supernatants were carefully collected and stored at –80 °C until further analysis.

Quantitative assessment of growth factors was performed using a sandwich enzyme-linked immunosorbent assay (ELISA). TGF-β and PDGF levels were measured using the Rat TGF beta-1 ELISA Kit Cat. No: BMS623-3 (Invitrogen, Massachusetts, USA) and PDGF-BB ELISA Kit Cat. No: ERA49RB (Invitrogen, Massachusetts, USA), respectively, following the manufacturer's instructions. All reagents were equilibrated to room temperature before being used in the analysis process. Standards, markers, and samples were added to microplate wells pre-coated with monoclonal antibodies specific to either TGF-β or PDGF. After incubation, enzyme-conjugated detection antibodies and substrate solutions were added sequentially. The colorimetric reaction was measured at 450 nm using a microplate reader. Growth factor concentrations were calculated by plotting the absorbance values against the respective standard curves, which were derived from known concentrations.

Table 1. Precondition and treatment of alopecia-like condition in rats.

Group (n = 5)	Code	Precondition	Treatment
Healthy control	K1	No	no
Negative control	K2	Alopecia-like conditions	0.9% NaCl (K2)
Positive control	K3	Alopecia-like conditions	5% minoxidil (K3)
Treatment	K4	Alopecia-like conditions	100 µL/kg hypoxia-induced MSC exosomes
Treatment	K5	Alopecia-like conditions	200 µL/kg hypoxia-induced MSC exosomes

Note: The preconditioning phase involved administering fluconazole at a dose of 15 mg/kg/day for seven days. Subsequent treatments were administered once daily, every morning, for 14 consecutive days.

Statistical Analysis

The TGF-β and PDGF concentration data were first tested for normality using the Shapiro–Wilk test and for homogeneity of variances across groups using Levene's test. Upon confirmation of these assumptions, a one-way analysis of variance (ANOVA) was conducted to determine overall differences among the treatment groups, followed by a Least Significant Difference (LSD) post hoc test. All statistical analyses were performed at a 95% confidence level, with a significance threshold set at $p \leq 0.050$, using IBM SPSS Statistics software, version 26.

RESULTS AND DISCUSSION

MSC Isolation and Characterization

Mesenchymal stem cells were successfully isolated from rat umbilical cords at 21 days of gestation, confirming the feasibility of umbilical-derived MSC culture in vitro. Morphological evaluation under a light microscope revealed that within 24 hours of culture under normoxic conditions, the cells adhered to the surface of the culture flask and exhibited a fibroblast-like, spindle-shaped morphology characteristic of MSCs (Figure 1). These elongated, bipolar cells were observed aligning in parallel or forming whirlpool-like colonies, a typical feature of early-stage MSC outgrowth.

The observed morphology is consistent with established criteria defined by the International Society for Cellular Therapy (ISCT), which states that MSCs must be plastic-adherent when maintained in standard culture conditions (Dominici et al., 2006; Viswanathan et al., 2023). Based on the early appearance, the cell's adherence capability, and the uniform spindle shape show that the mesenchymal lineage is in a healthy state of proliferation. These morphological characteristics provide essential confirmation to validate MSCs before conducting immunophenotyping analysis and differentiation assays (Viswanathan et al., 2023). Flowcytometry analysis further validated the MSC phenotype by examining mesenchymal markers, including high expression of CD90 and CD29, alongside low expression of hematopoietic markers CD45 and CD34 (Figure 2).

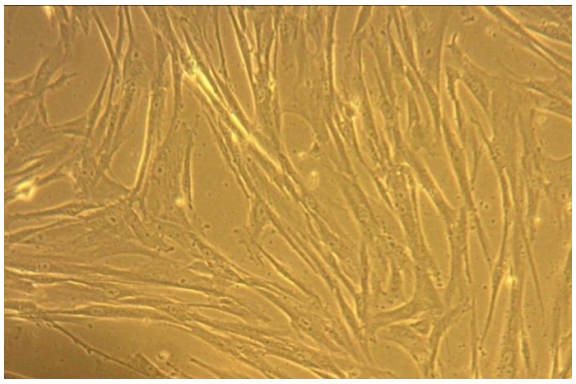

Figure 1. MSCs at phase 1 after 24 hours under normoxic conditions. Cell observation was performed using a light microscope at 400× magnification.

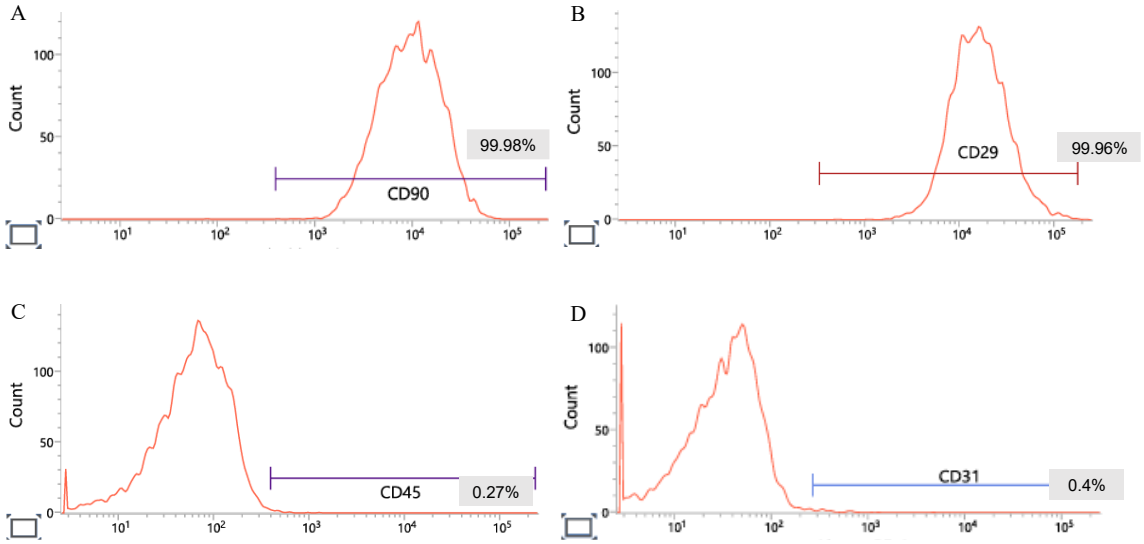

Figure 2. MSC phenotype confirmation is based on the high expression of the MSC surface markers CD90 (A) and CD29 (B), along with low expression of the hematopoietic and endothelial markers CD45 (C) and CD31 (D).

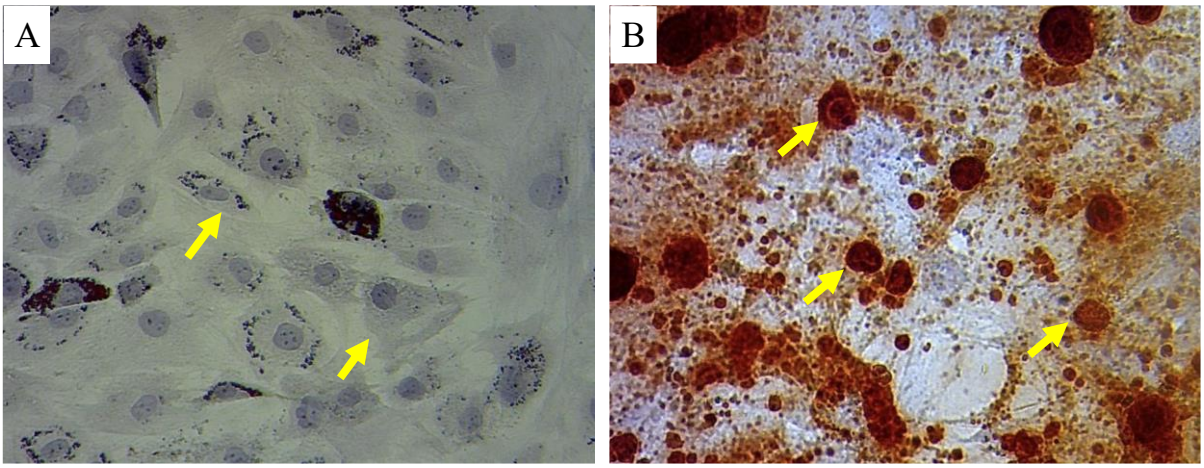

Figure 3. MSC differentiation capability. (A) Differentiation into osteocytes visualized with Alizarin Red staining and (B) differentiation into adipocytes visualized with Oil Red O staining. The differentiated cell is observed under a light microscope with 400x magnification (pointed by yellow arrows).

The purity of MSC was confirmed by the low expression levels of CD45 and CD34, which indicate the absence of hematopoietic lineage contamination in the cultured cell population (Shi et al., 2023). Furthermore, cell cultures that meet immunophenotyping criteria should be validated through differential assay analysis to demonstrate their functional multipotency (Allouh et al., 2025). The results of this study show that cultured cells also differentiate into multiple cell lineages, as illustrated in Figure 3.

Figure 3 demonstrates the multipotent differentiation capacity of the MSCs in this study. Figure 3A shows osteogenic differentiation, confirmed by Alizarin Red staining, which binds to calcium deposits formed in the extracellular matrix. The reddish-orange coloration, highlighted by yellow arrows, indicates the mineralization characteristic of mature osteocytes. Figure 3B illustrates adipogenic differentiation, visualized using Oil Red O staining, which selectively stains intracellular lipid droplets. The presence of prominent red-stained lipid vacuoles within cells, also marked by yellow arrows, confirms successful differentiation into adipocytes. These results collectively validate that the isolated MSCs possess the expected multipotency, further supporting their functional identity.

Exosome Isolation and Characterization

After validation, exosome levels were quantified using flow cytometry, revealing a concentration of 0.74 µg/mL. Further analysis showed that 28.2% of the bead-singlet population was positively labeled for both CD81 and CD63, indicating the presence of exosomes expressing these two major surface markers in significant proportions. This suggests that the majority of the captured and analyzed exosomes co-expressed CD81 and CD63, which are characteristic of endosome-derived exosomes (Figure 4).

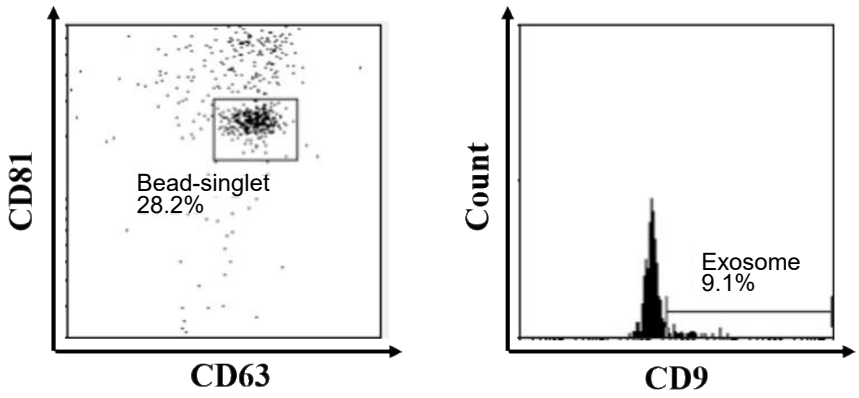

Figure 4. Exosome quantification based on flow cytometry analysis using the surface markers CD63 and CD9.9

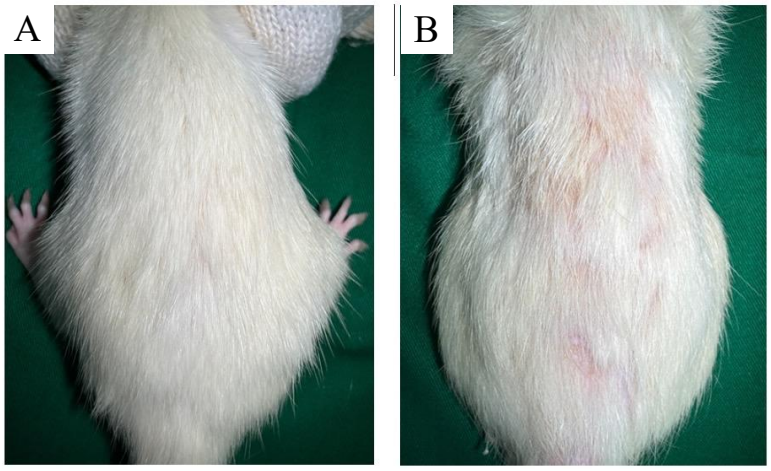

Figure 5. Validation of hair density in healthy and fluconazole-induced alopecia-like rats depicts a healthy rat with uniformly dense hair coverage, indicating normal follicular function (A). In contrast, rats exposed to fluconazole exhibited visibly reduced hair density consistent with an alopecia-like condition (B).

The exosome marker CD9 was detected at low expression, accounting for only 9.1% of the cell population. The low level of CD9 expression may vary depending on the cell of origin and the cell culture conditions during exosome production (Mathieu et al., 2021). Besides that, differences in antibody binding affinity or bead conjugation may affect CD9 detection compared to CD81 and CD63b markers (Khushman et al., 2017). Furthermore, the MSCs' internal cell regulation under hypoxic conditions may result in the release of subpopulations of exosomes with naturally lower CD9 expression. Nevertheless, the high expression of key tetraspanin markers CD81 and CD63 confirms the successful isolation of exosomes from MSCs (Fan et al., 2023). We assume that the lower expression of CD9 does not negate the characteristic of the isolated particles as exosomes; however, it may reflect the complexity and heterogeneity of extracellular vesicles produced by hypoxic MSCs.

Alopecia-like model confirmation

The validation of the alopecia-like model was performed visually by comparing hair density between healthy rats and rats induced with fluconazole. Visual observations revealed distinct morphological differences between the healthy rat and the fluconazole-induced alopecia model (Figure 5). The healthy rat exhibited dense and evenly distributed hair growth across the dorsal region, with no signs of thinning, hair loss, or dermal abnormalities. The underlying skin appeared smooth, intact, and free of redness, rash, or irritation, indicating optimal skin integrity and the absence of inflammatory responses.

The fluconazole-induced rat displayed clear characteristics of alopecia, including marked hair thinning, uneven hair distribution, and near-complete hair loss in some areas. Accompanying the hair loss were visible signs of erythema and irritation, suggesting the presence of localized inflammation. Although fluconazole is widely recognized as a systemic antifungal agent, evidence suggests that it may, under specific doses or durations, prematurely induce the telogen (resting) phase or inhibit the anagen (active growth) phase of hair follicles, thereby triggering diffuse hair shedding (Thompson et al., 2019). Additionally, the possibility of direct cytotoxic effects on follicular cells cannot be excluded, which could potentially impair the regenerative capacity of the hair follicles (Huang et al., 2022). The observed skin erythema and rash may also reflect an inflammatory response due to follicular damage or a hypersensitivity reaction to fluconazole in susceptible individuals.

PDGF and TGF-β levels

Hypoxia-induced MSC exosomes have emerged as a promising alternative therapeutic for regenerative and inflammatory conditions, including alopecia-like models. Particularly in this study, through their ability to upregulate TGF-β and PDGF expression. Based on the analysis, the concentrations of TGF-β and PDGF were measured across five experimental groups (K1 to K5). The results demonstrated statistically

significant differences among the groups (p = 0.000 for both factors), as shown in Table 1.

Table 1. Concentration of TGF-β and PDGF

Treatment group	Growth factors concentration (pg/mL)	
	TGF-β	PDGF
K1	36.33 ± 3.15 [a]	8.68 ± 0.40 [a]
K2	7.87 ± 1.38 [b]	4.12 ± 0.47 [b]
K3	17.93 ± 2.41 [c]	6.01 ± 0.18 [c]
K4	28.47 ± 1.64 [d]	7.42 ± 0.09 [de]
K5	34.14 ± 1.77[ae]	7.99 ± 0.44 [ae]
p-value	0.000	0.000

Note: Different superscript letters within a row indicate statistically significant differences between groups (LSD post hoc test, $p < 0.05$).

TGF-β levels varied markedly across the groups, with the healthy control group showing the highest expression, serving as a reference for normal physiological conditions. Among the treatment groups, administration of 200 µL hypoxia-induced MSC exosomes resulted in TGF-β and PDGF levels closest to the healthy baseline, suggesting a strong regenerative and anti-inflammatory effect. The dose of 100 µL hypoxia-induced MSC exosomes also led to a considerable increase in TGF-β levels and PDGF, indicating a dose-responsive relationship. In contrast, the control groups exhibited minimal TGF-β expression, reflecting the absence of therapeutic stimulation, while the group treated with Minoxidil showed only a moderate increase, suggesting a limited capacity to modulate this growth factor or immune mechanism.

These results collectively underscore the efficacy of hypoxia-induced MSC exosome therapy, particularly at the higher dose, in restoring both TGF-β and PDGF expression toward physiological levels. Elevated TGF-β levels in the 200 µL group indicate activation of pathways essential for skin and hair follicle regeneration, reducing alopecia symptoms. TGF-β suppressed pro-inflammatory T-cell activity, mitigating hair follicle damage (Moreau et al., 2022). Concurrently, increased PDGF levels promoted angiogenesis and skin regeneration, thereby enhancing nutrient supply and repairing follicle damage caused by chronic inflammation (Ji et al., 2021; Mao et al., 2022; Zhou et al., 2025). The findings highlight the multifaceted regenerative potential of MSCs in promoting tissue remodeling, modulating inflammation, and enhancing follicular repair in an alopecia-like model, surpassing the outcomes achieved with conventional treatments, such as minoxidil. Furthermore, these results are consistent with previous studies demonstrating that MSC-derived exosomes enhance the production of cytokines involved in hair follicle development and cycling, cellular proliferation, and angiogenesis—key processes that maintain a supportive follicular microenvironment and play a crucial role in promoting hair growth (Rahmanita et al., 2023).

Recent studies also explain that the therapeutic efficacy of hypoxia-induced MSC exosomes in alopecia has been attributed to their capacity to enhance the proliferation and migration of key follicular cells, such as dermal papilla cells and outer root sheath cells that are essential for hair growth and regeneration (Hu et al., 2024; Rajendran et al., 2022). Clinical applications of MSC-derived exosomes have demonstrated improvements in hair density, development, and reduced hair shedding. This result highlights the potential of MSC-derived exosomes as an innovative alternative treatment for alopecia (Dehghani et al., 2024). This result is also supported by a previous study using exosomes that are produced from human umbilical cord mesenchymal stem cells (hUMSCs). This exosome application offers practical advantages in treating alopecia due to its high efficacy in promoting hair regrowth (Hu et al., 2024; Jiao et al., 2024).

Previous studies have shown that hypoxia-induced MSCs' exosomes, enriched with bioactive molecules, are able to modulate hair follicle regeneration and promote hair regrowth. The exosome content includes miRNAs, such as miR-122 and miR-221, which regulate inflammasome-related pathways involving TGF-β and PDGF (Higashi et al., 2019; Zhang et al., 2023). Furthermore, miR-122 has been extensively studied in the context of tissue regeneration, fibrosis, and inflammatory gene pathways (Sun et al., 2018). Upregulated miR-122 also evidently activates the TGF-β signaling pathway, modulating inflammatory responses, and enhancing cell proliferation (Zhang et al., 2017). An increased level of TGF-β then inhibits pro-inflammatory T-cell activation, preventing hair follicles from inflammation-related damage and aiding tissue repair (Moreau et al., 2022). A similar function is exhibited by miR-221, which enhances PDGF gene expression and promotes cell proliferation, angiogenesis, and hair follicle regeneration. The increased production of PDGF triggers angiogenesis, improving blood supply and collagen synthesis, which are essential needed for

follicle damage recovery.

Additionally, a recent study reports the presence of miR-122-5p in MSC-derived exosomes, which counteract the inhibitory effects of dihydrotestosterone (DHT) on hair follicles. This miRNA targets the TGF-β1/SMAD3 signaling pathway and promotes follicular regeneration in androgenetic alopecia (AGA) (Liang et al., 2023). Furthermore, hypoxic conditioning also enhances the paracrine function of MSC-derived exosomes by upregulating miR-125b, a pro-angiogenic miRNA that supports endothelial cell survival and migration. This miRNA activity facilitates wound healing and maintains the vascular integrity of hair follicles (Zhang et al., 2021). Moreover, MSC-derived exosomes contain miR-378, which exerts its regenerative effects by modulating the Sonic Hedgehog (Shh) signaling pathway (Nan et al., 2021). Based on the previous results, we propose that the 200 µL injection dose may contain a higher concentration of exosomes, contributing to the greater delivery of several beneficial miRNAs that enhance TGF-β and PDGF production, suppress inflammation, facilitate hair follicle repair, and promote angiogenesis. The exosomal miRNAs may have the potential to highlight the broader regenerative capacity of MSC-derived exosomes, making them promising candidates for therapeutic applications in hair loss disorders. Hence, further studies should be conducted to understand the miRNA content in MSC-derived exosomes and their functional gene targets to enhance vascularization and follicular support. This work provides a novel perspective by linking hypoxia-conditioned MSC exosomes with enhanced TGF-β and PDGF expression in an alopecia-like model, offering new insights into their regenerative and immunomodulatory potential.

CONCLUSION

This study demonstrates that 200 µL of hypoxia-induced MSC-derived exosomes significantly enhances TGF-β and PDGF production levels in rats' skin. This result also suggests that hypoxia-induced MSC exosomes may have the potential to control inflammation, facilitate hair follicle repair and regeneration, and promote hair growth in alopecia. However, this study is limited by its focus on TGF-β and PDGF analysis only, excluding broader signaling pathways that are pivotal in hair follicle regeneration, such as NF-κB, Wnt/β-catenin, BMP, and Hedgehog. Further research is also needed to profile the contents of hypoxia-induced MSC-derived exosomes, particularly miRNAs that regulate target genes associated with inflammation through the miRNA-mRNA axis, as therapeutic agents for the treatment of alopecia.

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AUTHOR CONTRIBUTION STATEMENT

All authors contributed significantly to the work reported in this manuscript. YP conceived and designed the study, conducted the data analysis, supported data visualization, and led the manuscript writing. SPM was responsible for data interpretation and critically reviewed the manuscript for important intellectual content. ES assisted in the literature review and provided substantial feedback during the manuscript drafting process. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that artificial intelligence (AI) tools, including Grammarly and ChatGPT, were used solely for transliteration and language improvement purposes. The AI tools were not used for data collection, interpretation, data generation, and analysis in this manuscript; All of these procedures and steps were carried out entirely by the authors.

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