RESEARCH ARTICLE OPEN ACCESS

# Secretome-Based Therapy Promotes Epidermal Thickness Recovery and Follicular Regrowth in Fluconazole-Induced Alopecia in Rats

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Submission October 05, 2025
Accepted October 11, 2025
Available online on October 12, 2025



## ABSTRACT

**Background:** Alopecia involves hair loss characterized by decreased follicle density and epidermal thinning, which may be exacerbated by prolonged fluconazole exposure. The secretome derived from human umbilical cord mesenchymal stem cells (hUC-MSCs) possesses anti-inflammatory and regenerative properties that could aid follicular repair. **Methods:** Twenty-four male Wistar rats were randomly assigned to four groups: control, 5% minoxidil, hUC-MSC secretome, and secretome–minoxidil combination. Alopecia was induced by fluconazole administration for seven days, followed by a seven-day treatment period. Histological evaluation using hematoxylin and eosin staining assessed hair follicle count and epidermal thickness. **Results:** The hUC-MSC secretome group exhibited a significant increase in follicle count (mean 39.2; $p < 0.001$) compared with the control and minoxidil groups. However, changes in epidermal thickness were not statistically significant ($p = 0.133$). **Conclusion:** hUC-MSC secretome effectively enhances follicular regeneration in fluconazole-induced alopecia and represents a promising biotherapeutic approach for hair restoration.

**Keywords :** alopecia; hUC-MSC; secretome; hair follicle regeneration; epidermal thickness.

## INTRODUCTION

In general, alopecia is characterized by hair loss that can be either localized or total, often leading to significant psychological and physiological stress[1]. Studies have shown that prolonged fluconazole administration can induce marked hair loss, exceeding 25% compared to baseline conditions[2,3].

To date, the underlying mechanisms of fluconazole-induced alopecia (FRA) remain unclear, but it is hypothesized to involve inhibition of cytochrome P450 enzymes[4,5]. Fluconazole-mediated inhibition of cytochrome P450 reduces testosterone levels, thereby impairing hair follicle function and triggering alopecia[2]. In addition, fluconazole exposure may elevate pro-inflammatory cytokines, leading to oxidative stress, follicular damage, and disrupted hair regeneration[3].

Standard treatments for alopecia, such as finasteride and 2.5–5% minoxidil, are limited due to their slow therapeutic onset and frequent skin irritation. A promising alternative approach to address fluconazole-induced alopecia (FRA) involves the application of mesenchymal stem cell (MSC) secretome, which has the potential to modulate IL-15 and IFN-γ expression[6]. Preliminary studies using a rat model of fluconazole-induced alopecia demonstrated that topical administration of human MSC-derived secretome gel significantly reduced mRNA expression levels of the pro-inflammatory cytokines IL-15 and IFN-γ.

This reduction of pro-inflammatory cytokine expression correlated with a 60% decrease in baldness in the group treated with 20% MSC secretome[7]. Therefore, this study aims to evaluate the therapeutic effects of human MSC secretome on histological skin structure improvement, including epidermal thickness and hair follicle count, in a rat model of fluconazole-induced alopecia (FRA).

## MATERIAL AND METHODS

### *Study design*

This study employed an experimental design using a treated and control group. The research subjects consisted of 24 male Wistar rats aged 2–3 months with an average body weight of 200 g ± 10 g, obtained homogeneously from the Stem Cell and Cancer Research (SCCR) Center, Semarang, Central Java, Indonesia. The animals were kept under controlled environmental conditions, including a temperature of 20–24°C, humidity of 60%, a 12–14-hour light–dark cycle, and continuous ventilation. Throughout the 29-day experimental period, all rats were provided with standard feed exclusively, from the pre-treatment phase until termination.

### *Cell culture and hUC-MSC secretome collection*

Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) were obtained from the Stem Cell and Cancer Research (SCCR) Center, Indonesia. Cryopreserved hUC-MSCs were first expanded in two-dimensional (2D) culture using α-MEM supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin (PenStrep). The cells were cultured at 37˚C in a humidified incubator with 5% $CO_2$ until reaching passage 7 (P7).

For hypoxic conditioning, hUC-MSCs were incubated under 5% $O_2$ for 24 hours to stimulate the secretion of paracrine factors. Following hypoxic incubation, the culture medium (hUC-MSC-CM) was collected and centrifuged at 300 × g for 5 minutes at 4°C to remove cellular debris and residual particles. The clarified conditioned medium (CM) was then aliquoted, frozen, and stored at -20°C until further use in subsequent experimental procedures.

### *Animal Handling, Alopecia Induction, and Administration of hUC-MSC Secretome Therapy*

The topical gel formulation was prepared exclusively for the minoxidil-treated group. The gel was aseptically prepared by mixing a 5% minoxidil solution with a water-based gel base to achieve a final concentration of 200 mg per daily dose. Alopecia induction was performed by applying fluconazole to the dorsal skin of rats in groups K2, K3, and K4 from day 8 to day 14 to establish fluconazole-induced alopecia (FRA). Treatment began on day 15 and continued until day 22, with the following designations: Group 1 (CN) served as the negative control and received 0.9% physiological saline (NaCl); Group 2 (M) received 200 mg of 5% topical minoxidil gel once daily for 7 days; Group 3 (S) received intradermal injections of 100 µL hUC-MSC secretome into the shaved dorsal area; and Group 4 (SM) received a combination of 100 µL intradermal hUC-MSC

secretome and 200 mg/day of 5% topical minoxidil gel. Additionally, one healthy rat was used as a normal validation (V-SH), and one rat with fluconazole-induced alopecia without treatment served as a validation control (V-CN) for histological comparison.

On day 22, all experimental animals were euthanized via intramuscular injection of a lethal anesthetic mixture containing ketamine (6 mL) and xylazine (4 mL), administered at a total dose of 10 mL per animal. Dorsal skin samples were collected from the treated regions and preserved in RNA Iso Plus (Toyobo, Osaka, Japan) for gene expression analysis, while additional tissue samples were fixed in 10% formalin for histological examination. The remaining carcasses were disposed of through cremation at 850–1150°C for 30 minutes.

### *(HE) Hematoxylin-Eosin (HE) Staining*

Hematoxylin-Eosin (HE) staining of scalp tissue from fluconazole-induced alopecia rat models was performed following standard histological procedures. Skin tissues were fixed in 4% paraformaldehyde (PFA) solution for at least 15 hours at 4˚C, followed by embedding in Optimal Cutting Temperature (OCT) compound and rapid freezing using liquid nitrogen. The frozen tissues were sectioned at a thickness of 20-25 µm using a cryostat microtome and mounted onto glass slides. The sections were post-fixed in 4% PFA for 10 minutes at room temperature, rinsed, and subsequently stained with hematoxylin for 15 minutes and eosin–alcohol for 2 minutes, with gentle rinsing in water between steps. Gradual dehydration was carried out using a graded ethanol series (70%, 80%, 90%, and 100%), followed by two rounds of xylene clearing. The stained sections were examined under a light microscope to evaluate hair follicle and epidermal structures, and epidermal thickness was quantitatively assessed using microscopic image analysis.

### *Data Analysis*

All statistical analyses were performed using SPSS version 26 (IBM, New York, USA). The evaluated parameters included the number of hair follicles and epidermal thickness, with the latter measured using the EMegi analysis system. Data with normal distribution were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test to determine intergroup differences. Results were expressed as mean ± standard deviation (SD), and a p-value of less than 0.05 was considered statistically significant.

## RESULTS

### *Secretome Treatment Enhances Hair Follicle Regrowth in Fluconazole-Induced Alopecia*

Statistical analysis of hair follicle number revealed significant differences among the treatment groups. The dataset met the assumptions for one-way ANOVA, with normality confirmed ($p>0.05$) and homogeneity of variances verified by Levene's test ($p=0.271$). As shown in Figure 1., the mean follicle count varied considerably between groups, ranging from 1.0 in the validated negative control (V-CN) to 39.2 in the secretome-treated group (S). One-way ANOVA demonstrated a statistically significant effect of treatment on hair follicle number ($F = 16.09$, $p = 5.48 \times 10^{-7}$), suggesting that secretome administration markedly enhanced follicular regeneration in fluconazole-induced alopecia. The secretome-treated group (S) exhibited the highest mean follicle count, followed by the minoxidil group (M, 18.8) and the validated healthy control (V-SH, 16.6). Meanwhile, both the untreated alopecia control (CN, 8.0) and the combination group (SM, 8.4) showed minimal follicular recovery. These findings indicate that secretome monotherapy significantly promotes hair follicle regrowth, surpassing the efficacy of minoxidil and the combination treatment under the experimental conditions.

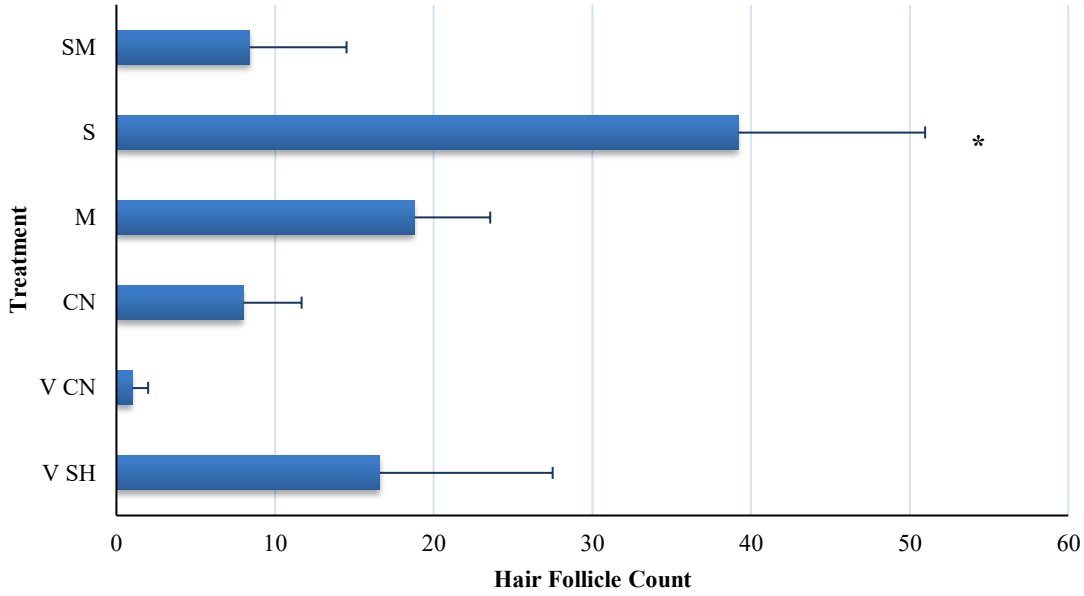


**Figure 1. Hair Follicle Count in Rats Treated with Secretome-Based Therapy after Fluconazole-Induced Alopecia.** (CN) served as the negative control and received 0.9% physiological saline, (M) received, 200 mg of 5% topical minoxidil gel, (S) received intradermal injections of 100 µL hUC-MSC secretome, (SM) received a combination of 100 µL intradermal hUC-MSC secretome and 200 mg/day of 5% topical minoxidil gel, (V-SH) healthy rat was used as a normal validation and V-CN) rat with fluconazole-induced alopecia as a validation control (V-CN). *: $p \leq 0.05$

## *Effect of Secretome-Based Therapy on Epidermal Thickness Recovery*

Quantitative analysis of epidermal thickness was performed to evaluate the effect of secretome-based therapy on skin recovery in fluconazole-induced alopecia. The mean epidermal thickness values (Figure 2) ranged from 33.16µm in the validated healthy control (V-SH) to 51.01µm in the validated negative control (V-CN). The secretome-treated (S) and combination (SM) groups showed slightly higher values of 49.02µm and 48.23µm, respectively, suggesting partial epidermal restoration. Data distribution was generally normal (except for group S), and variance homogeneity was confirmed by Levene's test (p=0.777).

However, one-way ANOVA demonstrated that the differences in epidermal thickness among all groups were not statistically significant (F=1.91;p=0.133), indicating that the secretome therapy did not induce a significant change in epidermal thickness. Nevertheless, the slightly higher mean values observed in the treatment groups may reflect early structural recovery, suggesting a potential dose or duration effect. Crucially, while no significant difference was observed in epidermal thickness, the secretome treatment showed a statistically significant difference in hair follicle count (as illustrated in Figure 1).

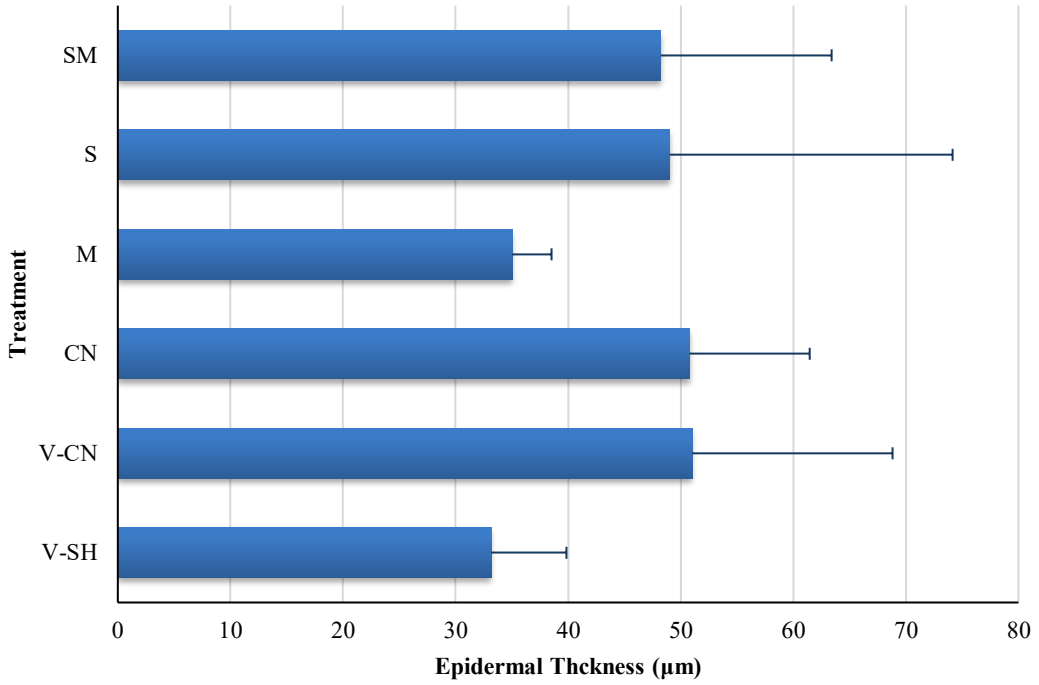


**Figure 2**. **Effect of Secretome-Based Therapy on Epidermal Thickness Recovery in Fluconazole-Induced Alopecia Rats.** CN) served as the negative control and received 0.9% physiological saline, (M) received, 200 mg of 5% topical minoxidil gel, (S) received intradermal injections of 100 µL hUC-MSC secretome, (SM) received a combination of 100 µL intradermal hUC-MSC secretome and 200 mg/day of 5% topical minoxidil gel, (V-SH) healthy rat was used as a normal validation and V-CN) rat with fluconazole-induced alopecia as a validation control (V-CN).

## *Histological appearance of rat skin*

The histological analysis of rat skin was performed to evaluate the structural changes in the epidermis and hair follicles following secretome-based therapy in fluconazole-induced alopecia. **Figure 1**. presents representative H&E-stained sections of rat skin on day 7 after treatment. The groups included the validated healthy control (V-SH), validated negative control (V-CN), untreated negative control (CN), topical minoxidil 5% (M), secretome treatment (S), and the combination of secretome and minoxidil (SM). Distinct morphological differences were observed among the groups. The secretome-treated and combination groups exhibited marked recovery of epidermal thickness and notable follicular regeneration compared to the alopecia control groups. The results demonstrate that secretome-based therapy promotes skin repair and stimulates follicular regrowth in this experimental model.

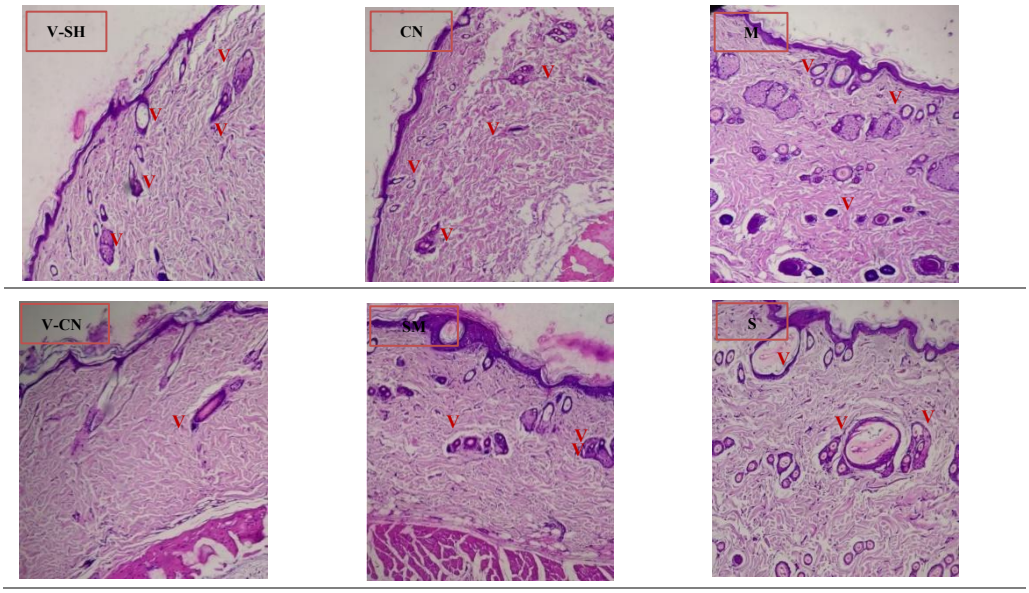


**Figure 3. Histological appearance of rat skin (Hematoxylin-Eosin staining) showing the effect of secretome-based therapy on epidermal structure and hair follicle regeneration in fluconazole-induced alopecia.treatment in day 7.** Validasi Sehat (V-SH), Validasi Control Negative (V-CN), control negative (CN), topical minoxidil 5% 200 mg (M), secretome (S) and combination of secretome and minoxidil (SM).

## DISCUSSION

This study evaluated the therapeutic potential of human umbilical cord–derived mesenchymal stem cell (hUC-MSC) secretome in a rat model of fluconazole-induced alopecia (FRA). Secretome treatment significantly increased hair follicle count compared to control and minoxidil groups, while epidermal thickness showed no significant difference among treatments. These findings suggest that the regenerative effect of the hUC-MSC secretome primarily acts through stimulation of follicular regeneration rather than epidermal remodeling during the 7-day treatment period. Similar observations have been reported in previous studies, where MSC-derived secretomes demonstrated potent regenerative effects through paracrine signaling rather than direct cellular replacement[8,9].

The observed follicular recovery supports the role of MSC-derived paracrine factors such as VEGF, FGF-2, IGF-1, cytokines, and extracellular vesicles in enhancing dermal papilla activity, reactivating quiescent follicles, and modulating inflammatory pathways[10,11]. These bioactive molecules are known to influence angiogenesis and activate signaling cascades including Wnt/β-catenin and PI3K/Akt, thereby promoting the transition of follicles from telogen to anagen phase[6,12]. The lack of synergistic effect in the combination group (secretome + minoxidil) may reflect pharmacological interference or insufficient treatment duration, as noted in previous studies evaluating combined topical therapies for alopecia[13,14]. Moreover, the absence of significant change in epidermal thickness likely represents an early stage of regeneration, which may require prolonged therapy for structural remodeling[15,16,17].

In contrast to the significant improvement in follicle number, epidermal thickness did not differ significantly among groups. Although minor variations were observed such as moderately increased mean values in the secretome and combination groups these changes were not statistically

significant ($p = 0.133$). This finding indicates that the structural recovery of the epidermis may lag behind follicular regeneration or require longer therapeutic exposure. Previous reports have shown that epidermal remodeling in response to MSC-based therapy typically occurs after sustained paracrine stimulation that enhances keratinocyte proliferation and extracellular matrix remodeling [9,18,19,20]. Therefore, the absence of significant epidermal thickening in this study may represent an early regenerative phase preceding full epidermal restoration.

Overall, these results underscore the potential of hUC-MSC secretome as a promising biotherapeutic agent for drug-induced alopecia. Its ability to promote follicular regeneration while exerting anti-inflammatory effects highlights its advantage over conventional pharmacological treatments[20,21,22]. However, the limited effect on epidermal thickness and the absence of synergism with minoxidil suggest that further optimization such as prolonged treatment duration, higher secretome concentration, or different delivery systems may be necessary to achieve comprehensive skin regeneration[23,24,25].

## CONCLUSIONS

This study demonstrated that human umbilical cord–derived mesenchymal stem cell (hUC-MSC) secretome effectively promotes hair follicle regeneration in a rat model of fluconazole-induced alopecia. Secretome monotherapy significantly increased follicle density compared to both control and minoxidil treatments, indicating its potent folliculogenic and regenerative activity. However, no significant improvement in epidermal thickness was observed, suggesting that epidermal remodeling may require longer therapeutic duration or higher dosing. Overall, hUC-MSC secretome represents a promising biotherapeutic candidate for managing drug-induced alopecia through its paracrine-mediated regenerative effects.

### Acknowledgement

We would like to thank the Stem Cell and Cancer Research (SCCR) Indonesia Laboratory for supporting our study.

### Competing Interests

There is no conflict of interest.

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