ORIGINAL ARTICLE

Comparison of Hair Follicle Density and Anagen:Telogen Ratio between Topical Minoxidil Alone and Its Combination with Platelet-Rich Plasma Exosomes in a Mice Model of Androgenetic Alopecia

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ABSTRACT

Introduction: Androgenetic alopecia (AGA) is a progressive condition driven by dihydrotestosterone (DHT)-mediated inhibition of the Wnt/β-catenin signaling pathway. While topical minoxidil is the first-line therapy, its efficacy is often limited by follicular sulfotransferase activity and patient non-response. The specific therapeutic potential of platelet-rich plasma (PRP) bioactive nanocarriers, such as PRP-derived small extracellular vesicles (sEVs) remains underexplored in combination protocols. This study aims to investigates whether the combination of PRP-derived sEVs and 5% minoxidil exerts a synergistic effect in a testosterone-induced AGA mouse model. **Methods:** An experimental study was conducted using 14 male BALB/c mice. AGA was induced via subcutaneous injection of testosterone (0.5 mg/day) for 10 days. Mice were randomized into two groups (n=7): Group A received topical 5% minoxidil twice daily, while Group B received topical minoxidil combined with weekly intradermal injections of PRP-derived sEVs (1×10^9 particles/mL) on days 11, 18, and 25. sEVs were isolated from calcium chloride-activated PRP using size-exclusion chromatography (SEC) and characterized by Nanoparticle Tracking Analysis (NTA). Histopathological analysis of hair follicle density (HFD) and anagen-to-telogen (A:T) ratio was performed on day 21. **Results:** The combination treatment (Group B) yielded a higher hair follicle density (120.82 ± 57.14 vs. 40.14 ± 11.69 follicles/mm²; $p < 0.001$) and a superior anagen-to-telogen ratio (0.54 vs. 0.22; $p < 0.001$) compared to minoxidil monotherapy. **Conclusion:** PRP-derived sEVs potentiate the hair-growth-promoting effects of minoxidil. This synergistic effect is likely mediated by the dual action of minoxidil-induced vascular support and sEV-mediated Wnt pathway activation.
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Keywords: Androgenetic alopecia, Small extracellular vesicles, Platelet-rich plasma, Minoxidil, Hair follicle regeneration

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INTRODUCTION

Androgenetic alopecia (AGA) represents the most common form of hair loss worldwide, affecting up to 80% of men and 50% of women by age 70 (1). The condition is characterized by progressive follicular miniaturization and a shortened anagen phase, driven by the conversion of testosterone to dihydrotestosterone (DHT). DHT binds to androgen receptors in the dermal papilla, triggering the release of catagen-inducing factors such as Dickkopf-1 (DKK-1), which inhibits the canonical Wnt/β-catenin pathway essential for hair growth (2).

Despite its prevalence, the therapeutic armamentarium remains limited. Topical minoxidil (2-5%) functions as an ATP-sensitive potassium channel opener, enhancing follicular blood flow (3). However, its efficacy is rate-limited by the expression of follicular sulfotransferase (SULT1A1), leading to non-response in approximately 30-40% of patients (4). This necessitates the development of adjuvant therapies with independent mechanisms of action.

Platelet-rich plasma (PRP) has emerged as a regenerative therapy, delivering growth factors like PDGF and VEGF to the follicular niche (5). While Elena and Irina (2022) demonstrated that combining whole PRP with minoxidil improves clinical outcomes (6), conventional PRP preparations are limited by variability in cellular content and the rapid degradation of growth factors (7). Recent advances highlight the superiority of cell-free therapies

using small extracellular vesicles (sEVs), historically termed exosomes (8). These lipid-bound nanovesicles (30–150 nm) encapsulate stable bioactive cargo, including miRNAs (e.g., miR-21-5p) and Wnt ligands, which can penetrate the follicular barrier and reactivate the Wnt/β-catenin pathway more effectively than whole cells (9).

There is currently a paucity of research directly comparing the synergistic efficacy of purified PRP-derived sEVs combined with minoxidil versus the standard of care (minoxidil alone) in controlled animal models. This study aims to fill this gap by evaluating the potential of this combination in a testosterone-induced AGA mouse model, utilizing rigorous isolation techniques (Size Exclusion Chromatography) to ensure high-purity sEVs.

MATERIALS AND METHODS

Study Design and Ethical Considerations

This laboratory-based experimental study employed a post-test-only control group design. The protocol was approved by the Health Research Ethics Committee of Dr. Moewardi Surakarta Hospital (Ref No. 1.853.B/X/HREC/2023). All procedures adhered to the 3Rs (Replacement, Reduction, Refinement) principle for animal welfare.

Fourteen healthy male BALB/c mice (8 weeks old, 20–25 g) were acclimatized for 7 days. BALB/c mice were selected due to the clear visibility of hair cycle transitions on their albino skin. To induce an AGA-like phenotype, mice received subcutaneous injections of testosterone enanthate (0.5 mg dissolved in 0.1 mL corn oil) on the dorsal skin once daily for 10 consecutive days for suppressing anagen entry, conceptually established protocol by Matias et al. (10).

Preparation and Characterization of PRP-Derived sEVs

PRP was prepared using a double-spin protocol to maximize platelet recovery and minimize leukocyte contamination. Whole blood from donor was centrifuged at 300 x g (10 min) to remove erythrocytes, followed by 2000 x g (10 min) to concentrate platelets. Platelets were activated with 10% Calcium Chloride (CaCl2). Unlike thrombin, which causes a rapid burst release, CaCl2 induces a sustained release of vesicles and growth factors, which is physiologically advantageous for hair follicle stimulation (11).

Subsequently, sEVs were isolated from the supernatant using Size-Exclusion Chromatography (qEV columns, Izon Science) to remove soluble proteins. In accordance with MISEV2023 guidelines, the particles were defined as small extracellular vesicles (sEVs) based on size (12). Nanoparticle Tracking Analysis (NTA) confirmed a mean particle size within the 30–150 nm range. The final concentration was adjusted to 1 x 10^9 particles/mL, a dose established in recent literature to elicit robust regenerative responses (13).

Treatment Protocol

Following the 10-day induction period, the mice were randomized into two groups, with seven mice in each group. Group A, serving as the control or monotherapy group, received topical 5% minoxidil at a dose of 0.1 mL twice daily. Group B, serving as the combination therapy group, received topical 5% minoxidil twice daily in combination with intradermal injections of PRP-derived small extracellular vesicles (sEVs) at a dose of 0.1 mL once weekly on days 11, 18, and 25.

Histopathological Analysis

On day 21, animals were anesthetized using chloroform in a controlled chamber. While isoflurane is the modern standard, chloroform was utilized in this specific protocol with ethical approval for rapid induction in a resource-limited setting, acknowledging its hepatotoxic limitations (14). Skin biopsies were fixed in 10% buffered formalin, embedded in paraffin, sectioned (5 µm), and stained with hematoxylin and eosin (H&E). Parameters assessed included Hair Follicle Density (HFD), defined as number of follicles per mm² and Anagen:Telogen (A:T) Ratio. Anagen follicles were identified by their location in the deep subcutis and fully formed inner root sheath (IRS), while telogen follicles were located in the upper dermis.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics 25.0. Results are expressed as mean ± standard deviation (SD). Differences were assessed using the Independent Samples t-test, with significance set at $p < 0.05$.

RESULTS

Histopathological evaluation on day 21 provided compelling evidence of the regenerative capacity of the combination protocol. Analysis of dorsal skin sections from Group A (Minoxidil 5% Monotherapy) revealed a moderate regenerative response. The follicular population was predominantly characterized by follicles situated in the upper to mid-dermis, with a limited presence of deep subcutaneous bulbs. The mean HFD was recorded at 40.14 ± 11.69 follicles/mm². This suggests that while Minoxidil initiates hair growth, its ability to fully overcome the androgen-mediated arrest is rate-limited. Representative histopathological images comparing hair follicle density between the minoxidil monotherapy and combination therapy groups are shown in Figure 1.

In contrast, Group B (Combination: Minoxidil + PRP-sEVs) exhibited a follicular awakening. Histology demonstrated an accumulation of enlarged, mature hair follicles deep into the subcutaneous adipose tissue (subcutis). Quantitatively, this group achieved a mean

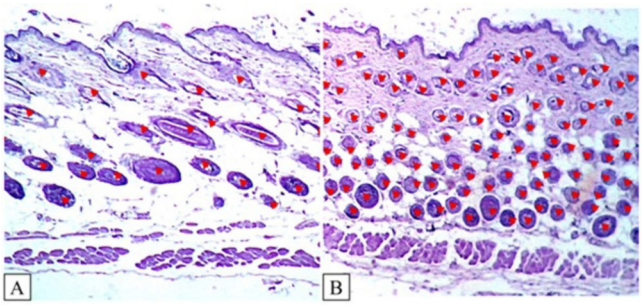

Figure 1: Histopathological comparison of Hair Follicle Density in dorsal skin tissue (H&E stain, 40x magnification). (A) Group A (Minoxidil 5% Monotherapy): The tissue section exhibits a moderate number of hair follicles, largely confined to the dermal layer with limited progression into the subcutis. (B) Group B (Combination: Minoxidil 5% + PRP-sEVs): The tissue section reveals a marked increase in the total number of hair follicles per field of view. The follicles are densely packed, enlarged, and exhibit varying stages of maturity. Red Arrowheads: Indicate individual hair follicles included in the density quantification.

HFD of 120.82 ± 57.14 follicles/mm².

The addition of PRP-sEVs resulted in an increase in follicle density compared to Minoxidil alone ($p < 0.001$) (Table I). This difference indicates that sEVs recruit a pool of dormant stem cells that Minoxidil monotherapy fails to activate.

Table I: Comparison of Hair Follicle Density (HFD) Between Treatment Groups on Day 21

Group	Mean HFD ± SD (follicles/mm²)	Range (Min – Max)	p-value
Group A (Minoxidil 5%)	40.14 ± 11.69	29.25 – 57.25	p<0.001*
Group B (Combination)	120.82 ± 57.14	88.75 – 248.50	p<0.001*

**Significant difference using Independent t-test ($p < 0.05$).*
Abbreviations: HFD,Hair Follicle Density; SD, Standard Deviation.

To assess the reversal of the androgenetic phenotype (miniaturization and telogen arrest), the A:T ratio was calculated. Group A presented an A:T ratio of 0.22, indicating that for every active follicle, approximately five remained in the resting (telogen) phase. This reflects a persistent lag in the hair cycle despite vasodilator therapy. Representative histopathological images showing differences in hair follicle morphology and anagen-to-telogen distribution are presented in Figure 2. Conversely, Group B demonstrated a superior A:T ratio of 0.54 ($p < 0.001$) (Table II). This shift signifies a tipping point where the regenerative signals provided by the PRP-sEVs overrode the inhibitory signals of testosterone, sustaining the anagen phase and preventing premature catagen entry.

DISCUSSION

This study demonstrates that combining PRP-derived sEVs with minoxidil yields an increase in hair follicle density compared to minoxidil alone. This finding aligns with recent studies by Zhang et al. (2025) and Liu et al. (2025), which identified exosome-mediated activation

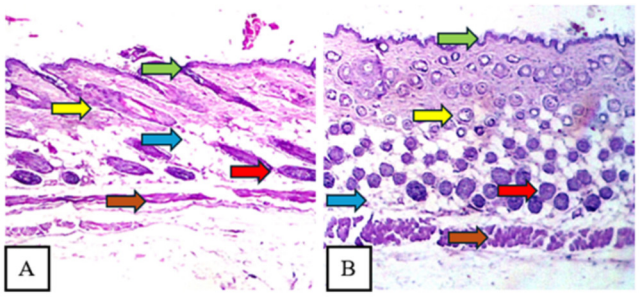

Figure 2: Histopathological evaluation of the Anagen:Telogen ratio and hair follicle morphology in dorsal skin tissue (H&E stain, 40x magnification). (A) Group A (Minoxidil 5% Monotherapy): The tissue section reveals a predominance of hair follicles in the Telogen (resting) phase or early Anagen. Follicles are smaller, less dense, and primarily located within the dermis. (B) Group B (Minoxidil 5% + PRP-sEVs): The tissue section demonstrates a shift towards the Anagen (growth) phase. Note the increased density of large, well-developed hair follicles extending deep into the subcutaneous adipose layer. Green Arrow: Epidermis, Yellow Arrow: Sebaceous glands and upper follicular structures in the dermis, Blue Arrow: Hair follicle shaft, Red Arrow: Hair Bulb, Brown Arrow: Panniculus Carnosus.

Table II: Comparison of Anagen-to-Telogen (A:T) Ratio Between Treatment Groups on Day 21

Group	Mean HFD ± SD (follicles/mm²)	Range (Min – Max)	p-value
Group A (Minoxidil 5%)	0.22 ± 0.02	0.20 – 0.25	p<0.001*
Group B (Combination)	0.54 ± 0.05	0.46 – 0.58	p<0.001*

**Significant difference using Independent t-test ($p < 0.05$).*
Abbreviations: HFD,Hair Follicle Density; SD, Standard Deviation.

of the Wnt/β-catenin pathway as a driver of anagen entry, capable of overriding DHT suppression (3,4).

The synergy likely stems from complementary pathways. Minoxidil acts primarily as a hemodynamic modulator. By opening ATP-sensitive potassium channels (KATP), it induces local vasodilation and upregulates Vascular Endothelial Growth Factor (VEGF), ensuring that the metabolically active hair bulb receives adequate oxygen and nutrients (15). However, Minoxidil does not directly antagonize the nuclear androgen signaling (DHT-AR complex) that suppresses the Wnt pathway (16). This likely explains the modest results in Group A where the blood supply was present, but the ignition signal was suppressed.

PRP-sEVs provide the missing ignition. Recent studies have elucidated that exosomes/sEVs carry a cargo enriched with Wnt ligands and specific miRNAs (e.g., miR-21-5p, miR-126) (3,4). These bioactive molecules penetrate Dermal Papilla Cells (DPCs) and inhibit the expression of DKK-1 (a Wnt antagonist upregulated by DHT) (3). This allows for the stabilization and nuclear translocation of Wnt/β-catenin, a regulator essential for anagen initiation and hair shaft differentiation (17). Furthermore, sEVs have been shown to modulate the mitochondrial apoptosis pathway by upregulating the anti-apoptotic protein Bcl-2 and downregulating the

pro-apoptotic protein Bax (18). This creates a survival shield that protects the follicle from testosterone-induced regression (catagen), explaining the superior A:T ratio observed in Group B.

Our findings refine the conclusions of previous clinical studies, such as Elena and Irina (2022), who reported improved outcomes with whole PRP and Minoxidil (6). By utilizing purified sEVs (1 x 10^9 particles/mL) isolated via SEC, our study demonstrates that the therapeutic potency of PRP is contained within its vesicular fraction. The transition to sEVs (cell-free therapy) offers advantages over whole PRP. Firstly, sEVs can be quantified and dosed precisely (particles/mL), overcoming the variability of platelet counts in whole PRP (18). Secondly, removing cellular components (leukocytes, RBCs) reduces the risk of pro-inflammatory reactions and immunogenicity (5). Finally, due to their nanometric size (30–150 nm), sEVs exhibit superior penetration into the perifollicular dermis compared to whole platelets (19).

Study Limitations

To ensure scientific rigor, we acknowledge specific limitations. First, this study utilized a head-to-head comparative design (Minoxidil vs. Combination) to evaluate the add-on benefit of sEVs. Future studies should ideally include a negative control (vehicle only) and a normal control to map the absolute baseline of spontaneous recovery. Second, while chloroform was used for anesthesia with ethical approval for this specific protocol, we recognize that isoflurane is the contemporary global standard for minimizing physiological stress, and future replication should adopt this refinement. Finally, molecular characterization relied on NTA and SEC, which future investigations should incorporate Western Blotting for specific exosomal markers (CD9, CD63, TSG101) to fully align with the evolving MISEV2023 guidelines.

CONCLUSION

The combination of PRP-derived sEVs and 5% Minoxidil represents a synergistic therapeutic strategy for Androgenetic Alopecia. This regimen does not merely add to the effects of Minoxidil but yielding an increase in hair follicle density and extending the anagen growth phase. Mechanistically, this synergy is likely driven by the convergence of Minoxidil-induced vascular recruitment and sEV-mediated reactivation of the Wnt/β-catenin signaling pathway, which collectively override the suppressive effects of androgens. These findings support the clinical translation of PRP-sEVs as a standardized, cell-free adjuvant therapy that addresses the limitations of current pharmacological options.

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