Original Article

CHALLENGING 100% PURITY: UNVEILING ALOE VERA'S OPTIMAL ROLE IN HAIR RESTORATION WITH INJECTABLE PLATELET-RICH FIBRIN

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ABSTRACT

This study investigates the effects of Aloe Vera, injectable platelet-rich fibrin (i-PRF), and their combination on the proliferation of mesenchymal stem cells (MSCs) derived from human hair follicles. MSCs were isolated using enzymatic digestion and characterized by flow cytometry for surface markers indicative of mesenchymal origin. The MTT assay was employed to assess cell proliferation and cytotoxicity at varying concentrations (0.01% to 1%) of Aloe Vera, i-PRF, and their combination. Aloe Vera alone showed limited proliferative effect at lower concentrations and significant cytotoxicity at 1% ($p = 0.0002$). In contrast, i-PRF significantly enhanced MSC proliferation at 0.1% ($p = 0.0028$) and 1% ($p = 0.0117$). When used in combination, Aloe Vera and i-PRF demonstrated a synergistic effect, with significantly increased MSC proliferation at 0.85% ($p = 0.00135$) and 1% ($p = 0.00243$). These findings challenge the prevailing notion that 100% purity or higher concentrations of Aloe Vera are inherently beneficial, emphasizing the need for optimal dosing. The study underscores the regenerative potential of combining plant-based bioactives with autologous biological agents and supports further exploration into their application for hair follicle regeneration and the treatment of androgenic alopecia.

Key words: Aloe Vera, i-PRF, Mesenchymal stem cells, Regenerative medicine, Androgenic alopecia.

Introduction

Male or female pattern baldness, also referred to as androgenic alopecia (AGA), is a widespread kind of hair loss that affects millions of people globally, irrespective of gender or ethnicity. In women, diffuse hair loss and, in men, a receding hairline are common initial signs of AGA. The necessity for effective treatment strategies is highlighted by the fact that, despite not immediately threatening physical health, AGA significantly influences emotional well-being and self-esteem [1].

Pharmacological treatments, particularly topical minoxidil and oral finasteride have been the mainstay of traditional AGA control strategies. These therapies have limits even though they are somewhat effective. A 5-alpha reductase inhibitor, finasteride, stops hair loss and encourages regrowth by lowering dihydrotestosterone (DHT) levels. However, because of the potential for adverse effects, including breast pain and sexual dysfunction, some patients can be deterred from continuous adherence [2].

Similarly, the vasodilator minoxidil is used extensively because it promotes hair growth. Its effectiveness varies from person to person, though, and results must be maintained by consistent use. The possibility of scalp irritation and unintentional hair growth in certain places may further complicate minoxidil's usefulness [3].

New therapy approaches have drawn interest because of their potential to treat AGA more thoroughly, given the limitations of existing therapies. The regenerative approach known as platelet-rich plasma (PRP) treatment, which employs growth factors from a patient's blood, has gained popularity due to its capacity to increase hair density and promote hair follicle regeneration [4]. However, PRP's efficacy can be variable, and it sometimes takes several sessions to get the desired effects.

As a more stable platelet concentrate that constantly releases growth factors, injectable platelet-rich fibrin (i-PRF) shows promise in regenerative medicine. Unlike traditional PRP, i-PRF does not require anticoagulants, maximising tissue regeneration and lowering the possibility of negative responses [5]. Despite its potential, i-PRF therapy alone may not always suffice to address the multifaceted nature of AGA.

Researchers have looked to complementary medicines with regenerative qualities after realizing the necessity for adjunct therapies to improve the effectiveness of i-PRF. Aloe vera is a well-known plant extract with medicinal properties that has gained attention for its anti-inflammatory and antioxidant properties. It is an interesting choice for adjuvant therapy in the treatment of AGA. The regeneration benefits of i-PRF may be enhanced by tocotrienol, an aloe vera component with anti-inflammatory qualities that can reduce oxidative stress [6].

Combining i-PRF with adjunct therapy like aloe vera can

potentially improve hair regeneration results in AGA patients. Researchers hope to increase hair follicle proliferation and reduce side effects by fusing the anti-inflammatory qualities of aloe vera with the regeneration capabilities of i-PRF, providing a more thorough approach to AGA care.

The Study investigates the potential synergistic effects of i-PRF and aloe vera on the proliferation of hair follicle-derived stem cells (HFSCs). By clarifying the complex relationships between these elements, researchers hope to learn new things about the pathophysiology of AGA and potential treatment approaches. The results of this study could lead to the creation of more individualised and efficient therapies for AGA. Filling a significant gap in the dermatological field and improving the lives of those impacted.

Materials and Methods

Isolation of mesenchymal stem cells from hair follicles
Using an enzymatic digestion technique, mesenchymal stem cells (MSCs) were extracted from human hair follicles. Following sterile collection, hair follicles were carefully cleaned using phosphate-buffered saline (PBS) enhanced with 1% antibiotic-antimycotic solution [7]. The follicles were then enzymatically digested using a solution containing dispase (2 mg/mL) and collagenase type I (1 mg/mL) at 37°C for 1 hour. The digested suspension was filtered through a 70-µm cell strainer to remove debris, followed by centrifugation at 1200 rpm for 10 minutes. The resulting cell pellet was resuspended in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution [8]. The cells were cultured in a humidified incubator at 37°C with 5% CO_2, and non-adherent cells were removed after 24 hours by changing the medium. The adherent cells were expanded until they reached 80–90% confluence before further experimentation. Hair follicles showed different rate of cell profileration at certain period under microscope. **(Figure 1)** Resultant mesenchymal stem cells well profound and separated from the hair follicles. **(Figure 2)**

Surface marker analysis of hair follicle-derived mesenchymal stem cells
To confirm the mesenchymal stem cell phenotype, surface marker analysis was performed using flow cytometry. Cells at passage 3 were harvested and resuspended in PBS containing 2% FBS. The expression of mesenchymal stem cell markers, including CD73, CD90, and CD105, was assessed using fluorophore-conjugated antibodies. Additionally, hematopoietic lineage markers such as CD34 and CD45 were analyzed to ensure the absence of hematopoietic contamination. The labeled cells were incubated at 4°C for 30 minutes in the dark, washed with PBS, and analyzed using a flow cytometer. Data were processed using FlowJo software, and cells expressing CD73, CD90, and CD105 while lacking CD34 and CD45 were identified as MSCs.

Cytotoxicity assessment of aloe vera, i-PRF, and their combination on mesenchymal stem cells
The cytotoxicity of Aloe Vera, injectable platelet-rich fibrin (i-PRF), and their combination at different concentrations was evaluated using the MTT assay. MSCs were seeded in 96-well plates at a density of 5000 cells per well and allowed to adhere overnight. The cells were then treated with varying concentrations of Aloe Vera (0.01%–1%), i-PRF (0.01%–1%), and a combination of Aloe Vera and i-PRF for 24 hours. After treatment, each well received 20 µL of MTT solution (5 mg/mL), which was then incubated for four hours at 37°C. Dimethyl sulfoxide (DMSO) was used to dissolve the formazan crystals, and a microplate reader was used to determine the optical density (O.D.) at 560 nm [9]. The percentage of cell viability in comparison to the untreated control group was determined. When assessing cytotoxic effects, a p-value of less than 0.05 was deemed statistically significant.

Results and Discussion

The effect of Aloe Vera on mesenchymal stem cell (MSC) proliferation varied across different concentrations. While lower concentrations did not produce statistically significant changes, a notable reduction in MSC proliferation was observed at 1% concentration ($p = 0.0002$). This finding suggests that higher doses of Aloe Vera may have a cytotoxic effect on MSCs **(Table 1)**.

In the i-PRF group, a significant increase in MSC proliferation was observed at 0.1% ($p = 0.0028$) and 1% ($p = 0.0117$) concentrations. These results indicate a dose-dependent stimulatory effect, with higher concentrations promoting cell proliferation more effectively. However, other concentrations did not exhibit statistically significant differences compared to the control **(Table 2)**.

When Aloe Vera and i-PRF were used in combination, a significant enhancement in MSC proliferation was noted at 0.85% ($p = 0.00135$) and 1% ($p = 0.00243$) concentrations. This suggests a possible synergistic effect, where the combined application of Aloe Vera and i-PRF at these concentrations leads to a more pronounced increase in MSC proliferation compared to their individual us. **(Table 3)**.

The present study investigates the combined effects of Aloe Vera and injectable platelet-rich fibrin (i-PRF) on mesenchymal stem cell (MSC) proliferation derived from hair follicles, challenging the conventional belief that higher purity of Aloe Vera equates to better therapeutic outcomes. Our findings suggest that Aloe Vera, when used at specific concentrations, may enhance stem cell proliferation, but higher concentrations can exert cytotoxic effects. Similarly, i-PRF demonstrated a dose-dependent stimulatory effect on MSCs, and its combination with Aloe Vera at optimal

concentrations exhibited a synergistic impact on cell proliferation. These results emphasize the importance of concentration-dependent modulation when utilizing natural and biological adjuvants in regenerative medicine.

Aloe Vera is widely recognized for its bioactive properties, including its anti-inflammatory, antimicrobial, and wound-healing capabilities [10]. However, its role in stem cell biology, particularly in hair restoration, remains underexplored. Our study demonstrated that Aloe Vera at lower concentrations did not significantly enhance MSC proliferation, while at 1%, a significant decline in cell viability was observed ($p = 0.0002$). This suggests that while Aloe Vera contains beneficial compounds, excessive concentrations may lead to cytotoxic effects, possibly due to an overload of bioactive compounds such as anthraquinones, acemannan, or polysaccharides, which can disrupt cellular homeostasis. These findings contrast with the common perception that higher purity or concentration of Aloe Vera always translates to improved therapeutic efficacy [11].

On the other hand, i-PRF, a rich autologous source of growth factors and fibrin matrix, significantly promoted MSC proliferation, particularly at 0.1% ($p = 0.0028$) and 1% ($p = 0.0117$). This aligns with previous studies highlighting the regenerative potential of i-PRF in enhancing cell viability, migration, and differentiation due to its sustained release of growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor-beta (TGF-β) [12]. The ability of i-PRF to stimulate MSCs in a dose-dependent manner reinforces its potential as a promising biological therapy for hair regeneration.

The most intriguing finding of this study was the synergistic effect observed when Aloe Vera was combined with i-PRF. The combination showed significant enhancement in MSC proliferation at 0.85% ($p = 0.00135$) and 1% ($p = 0.00243$), suggesting that Aloe Vera, when used in conjunction with i-PRF at optimal concentrations, can augment the regenerative potential of MSCs. This synergy may be attributed to Aloe Vera's anti-inflammatory and antioxidant properties, which complement the pro-regenerative effects of i-PRF, creating a microenvironment conducive to stem cell survival and proliferation [13].

While these findings present promising insights into the combined use of Aloe Vera and i-PRF in hair restoration, further studies are necessary to elucidate the underlying molecular mechanisms. Future in vivo studies and clinical trials are essential to determine the translational potential of this combination therapy in treating AGA and other forms of hair loss. Optimizing the formulation and delivery method could further enhance its clinical applicability.

Table 1. Mesenchymal stem cell proliferation in 3 different trials using Aloe Vera at different concentrations

Conc. in %	1st Trial	2nd Trial	3rd Trial	Mean	SD	P-value
0	0.1262	0.1212	0.1337	0.12703	0.00514	
0.01	0.1252	0.2212	0.1437	0.16337	0.04159	0.2873
0.02	0.2204	0.3522	0.1658	0.24613	0.07824	0.2570
0.05	0.2159	0.1841	0.2085	0.20283	0.01359	0.4837
0.1	0.1235	0.1565	0.1601	0.1467	0.01647	0.0205
0.5	0.2056	0.1715	0.2329	0.20333	0.02512	0.0560
0.85	0.2488	0.2575	0.2107	0.239	0.02032	0.1935
1	0.0294	0.0330	0.0114	0.0246	0.00945	0.0002

Note: SD=Standard Deviation

Table 2. Mesenchymal stem cell proliferation in 3 different trials using i-PRF at different concentrations

Conc. in %	1st Trial	2nd Trial	3rd Trial	Mean	SD	P-value
0	0.1223	0.1205	0.1351	0.12597	0.0065	
0.01	0.1423	0.1405	0.1551	0.14597	0.0065	0.0370
0.02	0.1563	0.2345	0.1684	0.1864	0.03437	0.1774
0.05	0.1991	0.1985	0.2057	0.2011	0.00326	0.5795
0.1	0.2772	0.2617	0.3024	0.28043	0.01677	0.0028
0.5	0.2491	0.2969	0.2938	0.27993	0.02184	0.9807
0.85	0.2003	0.2488	0.2232	0.2241	0.01981	0.0553
1	0.2835	0.3201	0.3001	0.30123	0.01496	0.0117

Table 3. Mesenchymal stem cell proliferation in 3 different trials using i-PRF along with Aloe Vera at different concentrations

Conc. in %	1st Trial	2nd Trial	3rd Trial	Mean	SD	P-value
0	0.1132	0.1111	0.1151	0.1131	0.00163	
0.01	0.1332	0.1311	0.1551	0.1398	0.01085	0.02638
0.02	0.1734	0.1408	0.1588	0.1577	0.01333	0.21557
0.05	0.2330	0.2084	0.1659	0.2024	0.02772	0.10865
0.1	0.2258	0.2223	0.2304	0.2262	0.00332	0.29552
0.5	0.2902	0.2089	0.2852	0.2614	0.0372	0.25269
0.85	0.6516	0.5382	0.6241	0.6046	0.0483	0.00135
1	0.3515	0.2497	0.2882	0.2965	0.04197	0.00243

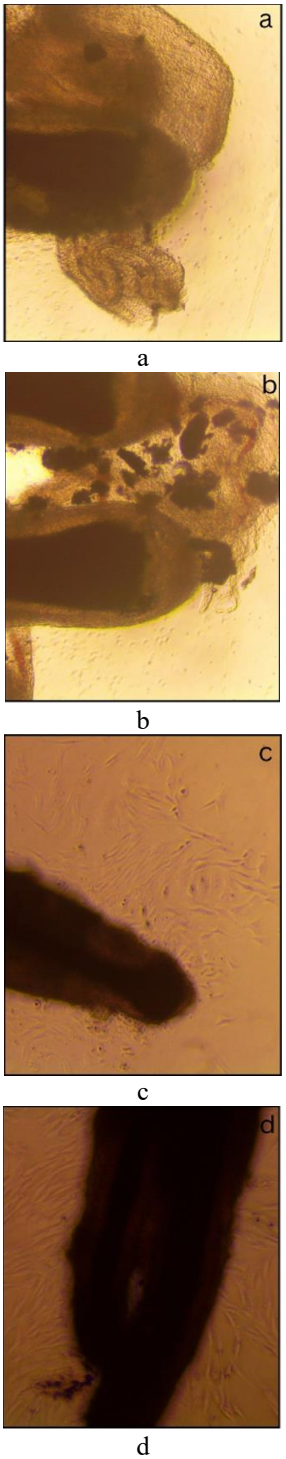

Figure 1. Hair follicle bulb observed under microscope at different intervals. a) Hair follicle bulb under microscope at day 0. b) The hair follicle bulb under microscope at day 1 showed initiation of cell proliferation. c) The hair follicle bulb under microscope at day 5 shows trace amount of cell proliferation. d) The hair follicle bulb under microscope at day 8 shows cell proliferation in abundance

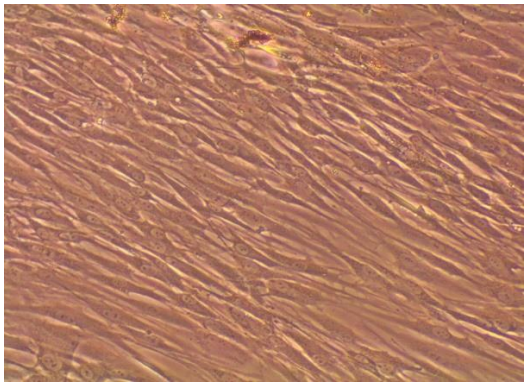

Figure 2. Isolated mesenchymal stem cells derived from hair follicles

Conclusion

In conclusion, our study challenges the prevailing notion that higher concentrations of Aloe Vera yield superior results, highlighting the importance of optimizing its use in regenerative medicine. When combined with i-PRF, Aloe Vera exhibits a synergistic effect, enhancing MSC proliferation and supporting its potential role in hair follicle regeneration. These findings pave the way for a more nuanced approach to natural and biologically derived therapies in hair restoration.

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