



# Regenerative Medicine Treatments for Androgenetic Alopecia

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## Abstract

### Keywords

- ▶ regenerative medicine
- ▶ nanofat
- ▶ plateletrich plasma
- ▶ stem cells
- ▶ androgenetic alopecia

Regenerative medicine and the role of stem cells are being studied for applications in nearly every field of medicine. The pluripotent nature of stem cells underlies their vast potential for treatment of androgenic alopecia. Several advances in recent years have heightened interest in this field, chief among them are the evolution of simpler techniques to isolate regenerative elements and stems cells. These techniques are easy, outpatient procedures with immediate injection, often single session with harvest, and minimal manipulation (usually physical). This paper seeks to critically review the existing data and determine the current evidence and their role in practice.

## Introduction

Regenerative medicine and the role of stem cells are being studied for applications in nearly every field of medicine. Hence, it was inevitable that they be viewed through the lens of hair loss treatment too.

The pluripotent nature of stem cells underlies their vast potential for treatment of androgenic alopecia (AGA).<sup>1</sup> Availability of autologous tissue makes it more attractive to practitioners and patients. However, traditional methods of obtaining stem wells were complicated, usually involving culturing. This meant that they were often expensive and could not be done in a single stage.

Several advances in recent years have heightened interest in this field, chief among them are the evolution of simpler techniques to isolate regenerative elements and stems cells. These techniques are easy, outpatient procedures with immediate injection, often single session with harvest, and minimal manipulation (usually physical). This also reduces cost and eliminates regulatory issues with the use of stem cells.<sup>2</sup> This has also led to awareness and publicity on social

media, demand by the patients, resulting in their clinical use, and even premature commercial exploitation. This paper seeks to critically review the existing data and determine the current evidence and their role in practice.

## Stem Cells, Growth Factors and Pathophysiology of Hair Loss

Management of alopecia involves modifications to two types of stem cells (SCs)—hair follicle stem cells (HFSCs), and dermal papilla cells (DPCs). In scarring alopecia, there is irreversible loss of these SCs. But in AGA and other non-scarring alopecias, these cells are preserved till late stage, which enables reversal.<sup>3,4</sup> The primary event in AGA is miniaturization of the HF. In balding scalp, the number of HFSCs remains intact, but the number CD34 and CD200 positive progenitor cells, which proliferate actively markedly decreases. This suggests that balding scalp may either lack an activator or has an inhibitor of HF growth.<sup>5</sup>

HFSCs express marker include CD34, K15, K19, CD200, PHLDA1, and EpCAM/Ber-EP4. DPCs express alkaline

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phosphatase, alphaSMA, laminin, fibronectin, and CD133.<sup>6,7</sup> These markers are modified in disease states. CK15 and CD34 both are lower, especially in the frontal scalp in AGA. SCs in the bulge remain in the resting phase for the vast majority of their lives. They are influenced by the mesenchymal cells of the dermal papilla and appear to be key in hair growth signal transmission. Early techniques of SC treatment for AGA involved isolating these SCs, culturing them, and then re-injecting them.

SCs are aimed at regenerating HFIs through various methods<sup>8–10</sup>:

- Reversing pathological mechanisms.
- Regenerating mature follicles from their parts, especially bulge cells.
- Neogenesis of HFIs in culture.

The main sources for stem cells include

- Bone marrow.
- Adipose tissue.
- Scalp tissue.
- Blood.
- Other tissues such as Wharton's jelly.

### Adipose Tissue-Derived Stem Cells

Normally, HFIs encompassed by subcutaneous fat cells and the dermis shape an interfollicular dermal macroenvironment, which is imperative for maintaining the best possible growth of bulge and follicle cells.<sup>11,12</sup> Adipocytes emit BMP2 during the late catagen stage and early telogen stage, which supports the resting state of HFSCs in the niche, whereas emission of BMP2 is lessened toward the late telogen stage, which bolsters the activation of HFSCs. Adipocytes thus play a critical role in extending the anagen stage.<sup>12,13</sup>

Adipose tissue (AT) contains numerous cells including adipocytes, adipose-derived stem cells (ADSCs), endothelial cells, fibroblasts, mural cells, and leukocytes.<sup>14</sup> The ADSCs have self-renewable capacity and display multilineage potential. The number of pluripotent cells contained in a cubic centimeter of adipose tissue is 100 to 1,000 times larger than the number of stem cells contained in bone marrow.<sup>15</sup> ADSCs also secrete various growth factors which have been shown to promote hair growth. These growth factors include vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), keratinocyte growth factor (KGF), and fibroblast growth factor-1 and 2 (FGF-1, FGF-2)<sup>16–21</sup>

ADSC-derived proteins improve hair growth and protect human DPCs against cytotoxic injury caused by androgen and reactive oxygen species. Moreover, conditioned media of ADSC (ADSC-CM) induces the anagen phase, promotes hair growth in mice, and enhances the elongation of hair shafts in ex vivo human hair organ cultures. ADSC-CM promotes hair growth in vitro, ex vivo, and in vivo.<sup>22</sup>

Thus, there seems to be a reasonable hypothesis for the use of ADSCs for AGA, based on the physiology. The technique is a simple and safe outpatient procedure and is well-

established in various other indications. Several different ways of harvesting and using adipose-derived cells have been described such as whole fat, nanofat, and stromal vascular fractions. Various commercial devices to extract them have been described, which are mentioned below.

### Stromal Vascular Fraction

The stromal vascular fraction (SVF) refers to all the cellular elements of the adipose tissue, excluding adipocytes such as smooth muscle cells, endothelial cells, blood cells, stem cells, and extracellular matrix.<sup>23</sup> The majority of activity of SVF is attributable to ADSCs, and there is continuous intercellular cross-talking and signaling between these cells.<sup>24</sup>

SVF can be obtained by two methods—chemical and mechanical. Chemical methods involve enzymatic digestion with collagenase, which is subsequently inactivated. The pool of mononuclear cells is then isolated through filtration and centrifugation. This chemical SVF can then be used to culture ADSCs.<sup>25</sup> Mechanical methods use physical disruption such as shaking, vortexing, sonification, etc., followed by washing and filtration. They contain fragments of extracellular matrix along with the cells.<sup>26</sup>

Mechanical SVF, also referred to as nanofat, has found increasing popularity, as it is easier to perform, requires less equipment, can be done in one sitting along with the harvest, and does not require any regulatory approval

Several studies have been published using the different types of fat mentioned above—autologous whole fat<sup>24–27</sup>, SVF, nano fat,<sup>28,29</sup> etc.<sup>27–36</sup> These have been tried in different types of alopecias such as traumatic scars, alopecia areata, scarring alopecias such as scleroderma, and also AGA.

ADSCs have been the most widely studied, most of which, however, are in small number of patients, of short duration of follow-up, and hence of low evidence quality. A number of commercial devices have become available for extraction of SVF. Early publications show some benefits with these devices.<sup>37,38</sup> One of the better studies with a long follow-up period was by Kuka et al who tried adipose-derived regenerative cell (ADRC)-enriched autologous fat grafts using Kerastem Celution system in 71 patients (17 females and 54 males) with AGA<sup>34</sup>. An increase in terminal hair count was demonstrated at weeks 6, 12, 24, and 52 weeks with maximum hair count seen at week 24. An interesting study by Butt et al assessed the efficacy of SVF combined with platelet-rich plasma (PRP) in 11 AGA patients. Mean hair density increased by 51.6% in SVF-PRP group as compared to only 21.5% in PRP group. There was also significant improvement in the physician and patient assessment scores in the SVF-PRP group.<sup>39</sup> A similar study by Stevens et al also reported benefits with the combined therapy of SVF with PRP (platelet-rich stroma [PRS])<sup>40</sup>

Several studies are available for ADSC-CM using commercial ADSC-CM products called advanced adipose-derived stem cell protein extract (AAPE) (Prostemics Co, Ltd, Seoul, South Korea) by Fukuoka et al who studied 68 patients of male and female pattern hair loss (PHL) over 5 years in three published studies.<sup>41–44</sup> ADSC-CM was found to be effective in

inducing hair growth. However, the authors seem to have recruited different patients in each study, and hence the follow-up period was not long in any of the studies, which is a significant limitation of these studies.

ADSCs have also been found to have efficacy in other pathological alopecias like AA.<sup>45-47</sup> The same has been reported in patients undergoing hair transplantation as well.<sup>47</sup>

In terms of evaluation, there is some evidence of improved outcomes with AT treatments. However, there was significant variation in the methods of preparation of ADSCs and fat from harvested AT. Till now, there is no universal protocol for fat grafting and the various methods of fat harvesting, processing, and reinjection can affect results which lead to difficulty in comparing results from various studies. Most of these studies had small sample size, variable control groups, and different durations, methodologies and outcome measures. No large controlled studies exist and the level of evidence is low. They also lacked long-term data of less than 6 months except the study by Kuka et al. Hence, it can be said that AT and ADSCs may represent a potential therapy for hair regeneration and physicians need to be aware of developments in this field. Hence, the modality cannot at this stage be recommended as a routine option for management of AGA and can only be tried as part of a scientific research.

### Follicle Derived Stem Cells and Hair Loss

Current evidence has shown that in balding scalp, the number of HFSCs remains intact, whereas the number of more actively proliferating progenitor cells markedly decreases.<sup>48</sup> This suggests that balding scalp either lacks an activator or has an inhibitor of HF growth. The authors Ruiz all developed a new method to isolate human adult stem cells by mechanical centrifugation of punch biopsy from human HFs. The scalp biopsy samples were mechanically disaggregated by a special centrifuge (Rigenera technology) to obtain an extract. The extract was directly infiltrated into the scalp of patients and patients were followed-up for 2 months. There was an increase in hair density and hair count.<sup>8,49</sup> However, these are the only two published studies on this topic, and it is too premature to make any recommendations on this limited data

### Platelet-Rich Plasma in Treatment of Hair Loss

PRP treatment has been steadily gaining popularity as a treatment option for hair loss, mainly AGA, in both men and women. PRP is the autologous plasma with platelet concentration, 2-7 times more than the baseline after centrifugation.<sup>50,51</sup> Graziani et al suggested that a PRP concentration 2.5 times the baseline is optimal and that concentrations above this level would be inhibitory in nature<sup>52</sup>

The activation of platelet  $\alpha$ -granules releases more than 20 different growth factors and cytokines, which are important in promoting cell proliferation and differentiation as shown in the box below.<sup>53,54</sup> These include PDGF, IGF-1,

transforming growth factor (TGF), endothelial growth factor (EGF), basic fibroblast growth factor (b FGF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and hepatocyte growth factor (HGF).

These growth factors have several actions on the HF, which are not fully understood as yet. These include:

1. Upregulation of GFs like IGF-1, FGF-7, HGF, and VEGF within the DPCs, thereby lengthening the anagen phase.<sup>55,56</sup>
2. EGF, TGF, and PDGF may upregulate the growth and differentiation of the bulge cells by GFs.<sup>56-58</sup>
3. Activation of Wnt/ $\beta$ -catenin pathway,<sup>56,59,60</sup> thereby promoting conversion from telogen to the anagen phase.
4. Activation of extracellular signal-regulated kinase (ERK) and protein kinase B (Akt) signaling that promotes cell survival and prevents apoptosis<sup>56,60,61</sup>
5. PRP may also work via other anti-inflammatory mechanisms, too, which are helpful in the treatment of alopecia areata<sup>62,63</sup>

These actions have led to the use of PRP in a number of clinical situations. However, it is also true that some actions of different growth factors may be conflicting in different situations and hence needs to be interpreted carefully.

Published date on the use of PRP in AGA has been expanding over years<sup>53,64-77</sup>. Several studies of use of PRP in AGA have been published with varying results in improvement in hair count, hair diameter, patient satisfaction, etc.

A critical analysis of these studies shows one striking aspect: the lack of standardization and wide variability in every aspect of PRP preparation, administration, and assessment. There is variation in volume of blood, type and duration of spin, type of kit and activator used, frequency, duration and follow-up period, etc.

- a) Volume of blood collected: As stated earlier, the platelet count in PRP should be at least 3 times the normal to be significant. This means that at least about 25 to 30 mL blood would need to be collected. However, volumes of blood collected for preparation of PRP by different authors have ranged from 3.5 mL to 478 mL, all demonstrating beneficial outcome! Some studies have used lower volumes of blood up to 5 mL, with a smaller yield ranging around 1 to 2 times the baseline platelet count.<sup>53,67,74</sup> While higher volumes of blood have yielded higher platelet concentrates of 4 to 6 times the baseline,<sup>65,66,69,71</sup> there are a few exceptions of lower yields with higher blood volumes<sup>70,75,76</sup>. Vice versa is also true, with one study showing higher concentrate from a lower volume.<sup>68</sup>
- b) Single versus double spin: Various authors have used various methods of preparation which may involve double- or single-spin centrifugation. Studies with single-spin centrifugation yielded only 2 to 3 times the baseline platelet concentrates even with higher volumes of blood collected.<sup>75,76</sup> Double-spin methods have generally yielded higher platelet concentrates with less amount of whole blood drawn as compared

to single spin<sup>34</sup> and hence may yield better outcome.<sup>78</sup> There is inconsistency in the use of centrifugation force expressed as “rpm,” rotations per minute and “g,” relative centrifugal force, in different studies.<sup>79</sup> Use of RPM can often be misleading, as it varies with the machine used and depends on the radius of the machine which makes replicating the findings of these studies difficult.

- c) **Methodology:** Methods of outcome assessment in different studies have varied widely, making the interpretation difficult. Global photographic assessment was found to be the most common method of evaluation of hair growth. While this was done every session by some,<sup>80</sup> others took photographs only at baseline and at 3- or 6-month intervals.<sup>81–83</sup> Only few studies used dermoscopy, trichogram, and hair count for assessment of improvement.<sup>65,75,80</sup> Further, these studies lacked adequate data on the photographic techniques used to reproduce the precise conditions in subsequent sessions.<sup>65,82,84</sup> Only few studies have used special methods such as semipermanent tattoos, fixed stamps, and easy to remember landmarks to avoid the above variabilities.<sup>75,80,81,85</sup>
- d) **Number of sessions, duration of treatment and follow-up:** There has been no uniformity in the number of session and interval between the sessions. Most studies used 3 sessions at monthly intervals. One of the most important yardsticks for any treatment of AGA is the duration of treatment and follow-up period. Majority assess the outcome by the end of the study<sup>81,84,86</sup> which range from 3 to 6 months, depending on the number of treatment sessions. This can lead to premature conjecturing as changes and effects may be delayed. Follow-up assessment of outcome a few months after cessation of therapy is available only in few studies.<sup>75,82,83,85</sup> Duration of follow-up is short, less than 1 year in most studies.
- e) **Type of kits:** The initial PRP studies used preparation of PRP without any kit. Subsequent studies have used commercial PRP systems, which facilitate the preparation of PRP in a reproducible manner with a smaller volume of drawn blood. They use different activators and gels to aid separation of different fractions of blood. Assessment of these kits is difficult as they differ markedly in their ability to collect and concentrate platelets as well as in the method and time of centrifugation.<sup>7</sup>
- f) **Placebo Component in PRP:** PRP as a treatment has a strong placebo component. Use of one's own blood is an impressive concept on the impressionable mind of an AGA patient, looking desperately for a magical cure! Puig et al in their double-blinded placebo controlled study showed that PRP treatment showed no statistically significant improvement when compared with placebo saline injections.<sup>84</sup> They concluded that PRP might have a strong placebo effect due to reporting of improvement of hair loss and texture by the study subjects as well as the

placebo group. Another study by Ayatollahi et al showed no favorable results at the end of 5 sessions.<sup>81</sup> An important feature, which explains its popularity, is that PRP has been founded to be relatively free from side effects, except minor side effects such as pain, discomfort, or mild scalp pruritus.<sup>50,85–88</sup>

Thus, it is obvious that a proper assessment of different studies is fraught with difficulty. While the above stated limitations are significant and relevant, it cannot be denied that there are multiple published studies that document efficacy of PRP and provide some evidence supporting PRP therapy in AGA with significant increase in number of hairs per cm<sup>2</sup> with minimal to no adverse effects.<sup>89</sup>

A study by Schiavone et al on 64 patients with AGA treated with PRP showed some form of improvement in 97.5% of the patients. This included increase in hair thickness as well as hair density. They observed no significant difference clinically based on the gender or age group and concluded that PRP treatment was useful in both male and female PHL.<sup>82</sup>

Gupta et al performed a meta-analytic study which concluded that PRP could be used to improve hair restoration parameters (e.g., hair density) as a monotherapy or as an adjunct therapy.<sup>88</sup> PRP has been shown to work even in patients who did not respond to topical minoxidil.<sup>80</sup> A well-designed randomized double-blinded placebo control study from India published recently (2020) on 80 patients with AGA compared the efficacy of different modalities in four groups (topical minoxidil only, PRP with minoxidil, normal saline [NS], and PRP only) in the treatment of AGA. The study found that the combination therapy of PRP with minoxidil was superior to all the other modalities. An interesting finding was that PRP as monotherapy was found to be superior to topical minoxidil in terms of hair density increase and patient satisfaction<sup>85</sup> Similar results have been noticed in studies by Verma et al and Vats et al.<sup>86,89</sup>

Thus, the above review shows that literature on efficacy, preparation, administration, and treatment regimens are conflicting, and several questions still need to be answered. Further, high-quality controlled studies are required to accurately predict its long-term efficacy. It is significant that these questions persist despite PRP being available for nearly 5 years in the field of hair loss. It is significant to note that the European guidelines for AGA<sup>7,90</sup> classified the evidence available as level 3 (studies grade B evidence or studies with mainly consistent results grade C evidence), and stated efficacy to improve or prevent hair loss as doubtful. The guidelines concluded that “there is no standardized technique for performing PRP to permit objective evaluation of its effects on AGA. We cannot make a recommendation for or against treatment of AGA with PRP at the present time. The impact of PRP treatment on quality of life of the patients should be further investigated.” In the authors' opinion, there is a void in the management of AGA between drug therapy and surgical therapy and PRP has sought to fill this void. However, there is a need to answer the following requirements, if PRP has to establish its position as a routine effective option:

1. Standardized method of preparation with agreement on the minimum level of platelet count needed and identification of precise growth factors and their levels in the PRP.
2. Standardized frequency and duration of administration.
3. Studies on long term efficacy over 3 to 5 years.

The precise role of PRP, till then, will continue to be debated.

## Summary

Regenerative treatments in management of hair loss has attracted great attention, both of doctors and lay public. However, the evidence shows that the potential and promise are yet to be met. Till standardization is achieved, controlled trials establish the evidence, and long-term data available, their routine use in clinical management is debatable. Evidence-based medicine demands that physicians adapt a questioning attitude and adhere to strict protocols while adapting such treatments. Below guidelines<sup>91</sup> are recommended whenever physicians use such treatments:

1. Use such treatments only when standard treatments fail.
2. Full explanation should be provided to the patients about all aspects of the treatment and available evidence, specifically mentioning that adequate evidence is not yet available for the treatment, as part of informed consent.
3. Proper documentation about efficacy and safety should be ensured.
4. Cost of such treatments should be reasonable.
5. There should be no advertisements for such treatments.

### Conflict of Interest

None.

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