

Research Article

Stem Cell Therapy in Androgenetic Alopecia: A Review of Dermatology Literature from 2012-2022

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Abstract

Androgenetic alopecia is considered the most frequent form of progressive hair loss. In this disease, an alteration of the hair cycle dynamics leads to progressive miniaturization of the hair follicle and possible baldness as a final outcome. A few treatments have been proposed to reduce the progression of hair loss. In this study, a search for relevant literature was performed using PubMed. We reviewed about 165 articles from 2012 to 2022 using the keywords "regenerative medicine", "stem cells" and "androgenetic alopecia". As a result, we described the hair follicle cycle, assessed how a change in its homeostasis can impact androgenetic alopecia and investigated the advent of new therapeutic techniques for hair regrowth, highlighting the use of stem cells and its impact on androgenetic alopecia prognosis.

Keywords: Androgenetic Alopecia; Stem Cells; Regenerative Medicine

Introduction

Androgenetic Alopecia (AGA) is considered the most common group of nonscarring alopecia and also the most frequent form of progressive hair loss [1,2]. AGA can affect all races, but prevalence rates vary. The prevalence is considered highest in Caucasians, with prevalence rates of around 30% for men in their 30s, 40% for men in their 40s and 50% for men in their 50s [3]. For Indians, the prevalence rate reaches 58% of AGA in men aged between 30 and 50 years [4]. In Oriental races, the prevalence is lower, around 21.3% for Chinese and 14.1% for Koreans [5].

Epidemiological studies of AGA in women are less numerous, with studies showing 19% in Caucasians, [3] 6.0% in Chinese and 5.6% in Koreans, suggesting that, as in men, the prevalence is thought to be lower in Oriental races compared to Caucasians [3,5]. The incidence of AGA in women also tends to increase with age [6]. It should be noted that experts have suggested that

female androgenetic alopecia is not exactly the female counterpart of male AGA. A better term for female AGA would be "female pattern alopecia" or "female pattern hair loss". The clear difference in the clinical pattern of male and female pattern hair loss suggests that these are two separate entities. This is also based on studies that have shown no clear relationship between excessive testosterone levels and female pattern hair loss [6]. In order to reduce the progression of hair loss, a few treatments have been proposed, such as 5 α -reductase inhibitors (finasteride, dutasteride), androgen receptor antagonists, androgen-independent therapies, coadjuvant therapies and emerging therapies, such as Platelet-Rich Plasma (PRP) injections, scalp micro-needling and others [7]. In this context, the use of Stem Cells (SCs) gained notoriety.

In this review, we describe the Hair Follicle (HF) cycle and discuss how a change in its homeostasis can impact diseases like AGA. We also investigate the advent of new techniques for hair loss, highlighting the promising use of SCs for AGA's treatment and prognosis.

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Methodology

Due to the growing interest in hair regeneration therapy, many studies involving new treatments for AGA have been performed. In our study, a search for relevant literature was conducted using PubMed. A combination of the keywords “regenerative medicine and stem cells and androgenetic alopecia” and “regenerative medicine and androgenetic alopecia” were used and all publications from 2012 to 2022 were analyzed; in total, about 165 articles were reviewed. We selected studies involving hair regeneration therapies for AGA and excluded studies unrelated to the domain of dermatology and hair loss; additionally, works involving other types of diseases other than AGA were also discarded. We also analyzed articles that described the HF cycle and AGA disease to give us a general overview of the context behind the advent of new options for hair regeneration for AGA patients. Some of the studies that we analyzed in this review are summarized in Table 1.

Authors	Study Type	Substrate Technique	Characteristics of the participants (Completed Study)	Objective Measures	Objective Assessment of Hair Growth	Year	Reference
				To study the prevalence and types of AGA in China and to compare the results			
Wang, et al.,	-	Population-based study	Main group: 15 257 M, Age range 18-70 with AGA	with those previously reported in Caucasians and in other Asian people.	-	2010	[3]
			Main group: 21 (21) 16M and 5F, age range 27-69 years, with AGA	1. Hair count on the main study	1. Yes, increase of $32 \pm 12,1$ at 3 months ($p < 0.01$)		
Fukuoka;	Half-head	ADSCs	Half-side comparison group: 10 (10) 8M, 2F, age range 20-73 years, with AGA	2. Hair count on the half-side comparison study	2. Yes, increase on both treated (ADSC-CM) and placebo sides, but significantly higher on the treated one (18.4 ± 9.4 , $p < 0.01$)	2017	[8]
Suga		(ADSC-CM)	40 (40)	1. Mean hair density	1. Yes, increase ($p < 0.01$) at 6 months 2. Yes, increase ($p = 0.022$) at 6 months		
Narita, et al.,	-	ADSCs	21M, 19F, age range 23-74 years, with AGA	2. Anagen hair rate	3. Yes, increase ($p = 0.049$ and $p = 0.003$) at 4 and 6 months, respectively	2020	[9]
		(ADSC-CM)		3. Dermal thickness 4. Dermal echogenicity	4. Yes, increase until 4 months ($p = 0.022$); decrease from 4 to 6 months ($p = 0.001$)		
				1. Hair density in temporo-parietal region	1. Yes*, increase of 10-20% in 75% of the male patients and 10-20% in 100% of the female patients, both at month 3 2. Yes*, increase of 10-20% in		

					100% of the		
Öztürk;	-	ADSCs (SVF treatment)	20 (20) 14M, 6F, age range 21-41 years, with AGA	2. Hair density in vertex region	male patients and 10% in 100% of the female patients, both at month 3	2020	[10]
Bekerecioğlu				3. Hair thickness in temporo-parietal region 4. Hair thickness in vertex region	3. *Increase of 25% in 100% of the male patients, but no change in 100% of the female patients, both at month 3 4. *Increase of 10-30% in 50% of the male patients, but no change in 100% of the female patients, both at month 3		
			71 (60) 54M, 17F, age range 24-73 years, with AGA	1. Nonvellus (terminal) hair count	1. Yes, increase for the low-dose ADRC group in the Norwood Hamilton 3 subgroup at week 24 when compared to no-fat saline control (p = 0.0318)		
		ADSCs (ADRC-enriched)	Patients were assigned to receive a fat graft	2. Total hair (nonvellus + vellus) count	2. Yes, increase for the low-dose ADRC group in the Norwood Hamilton 3 subgroup at week 6 (p = 0.0219) and 12 (p = 0.0434),	2020	[11]
Kuka, et al.,	Randomized Controlled	autologous fat)	cell enriched with	3. Nonvellus (terminal) hair width	and for the high-dose ADRC group in the Norwood Hamilton 3 subgroup at week 6 (p = 0.0465).		
			ADRCs (low or high doses), a fat graft without cell enrichment (fat alone control) or a saline injection (no-fat control)	4. Total hair width	3. Yes*, increase for the low-dose ADRC group in the Norwood Hamilton 3 subgroup at week 6, 12 and 24. 4. No, at any timepoint no statistical differences were observed between any of the treatment groups (p > 0.05).		
			22 (22) 17M, 5F, age range 21- 39 years, with AGA		1. Yes, increase of 21.51% in PRP group		
			Patients were divided		and 51.64% in SVF-PRP group after 6 months of last treatment (p = 0.006)		
Butt, et al.,	Randomized Controlled	ADSCs (SVF treatment)	into 2 groups: in PRP group, only PRP was injected; in SVF-PRP	1. Hair density 2. Number of pulled hair	2. Yes*, decrease in both groups after 6 months, more significant in SVF-PRP group (80.78 ± 5.84) as compared to PRP group (34.01 ± 22.44)	2020	[12]

			group, a mixture of PRP and SVF was injected in affected scalp areas				
			38 (34) 29M, 9F, age range 18-		1. Yes, increase of 19.2% in treatment group		
Tak, et al.,	Randomized Controlled	ADSCs (ADSC-CE)	59 years, all with AGA	1. Hair count	at 8 weeks (p = 0,002) and 28.1% at 16 weeks (p = 0.008)	2020	[13]
	Blinded		Patients were divided into 2 groups: control and treatment group	2. Hair thickness	2. Yes, increase of 14.2% in treatment group at 16 weeks (p = 0.026)		
			Main group: 22 (22) 11M, 11F, age range 20-70 years, all with		1. Yes, increase in all patients (29 ± 4.1 in		
Fukuoka, et al.,	Half-head	ADSCs (ADSC-CM)	alopecia Half-side comparison	1. Hair count on the main study 2. Hair count on the half-	male and 15.6 ± 4.2 in female) (p > 0.05) 2. Yes, increase on both left (treated with ADSC-CM) and right (placebo) sides, but	2015	[14]
			group: 10 (10) 8M, 2F, age range 20-73 years	side comparison study	significantly higher on the left (p < 0.05)		
Elmaadani, et al.,	Randomized	Autologous	40 (40), age range 10-50 years, 20 with resistant AA (7M, 13F) and 20 with resistant AGA (8M, 12F)	1. Assessment of clinical efficacy of BMMCs and FSCs therapies 2. Dermoscopic examination 3. Digital dermoscopic	1. Yes, BMMC and FSC techniques reached their maximum improvement after 6 months in AGA and AA patients, with no statistically difference between both methods (p = 0.426). There was significant improvement in females compared to males after BMMC and		
		BMMCs and autologous	Patients were divided into 4 groups: groups 1	examination 4. Assessment of efficacy	FSC treatment (p = 0.016 and p = 0.008 respectively), with no significant difference	2018	[15]
		follicular stem cells (FSCs)	(10 resistant AA) and 3 (10 AGA) received injections of	of BMMCs and FSCs therapies with histopathological examination of the scalp after the treatment	between both therapies 2. No, no statistical differences were observed for AA or AGA lesions (p > 0.05)		

			BMSCs; group 2 (10 resistant AA) and group 4 (10 AGA) received injections of FSCs		3. Yes, improvement for AA and AGA lesions ($p < 0.001$ and $p = 0.003$). 4. Yes*, increased immunoreactivities with CK 15, CD34 and CD200		
				1. Microscopic identification of HFSCs 2. Hair density			
Gentile, et al.,	-	HFSCs	11 (11) 11M, age range 38-61 years, with AGA	Parameters were evaluated with photographs, global assessment scores and immunocytochemistry	1. Yes*, $5\% \pm 0.7\%$ of HFSCs CD44+ (from dermal papilla) and $2.6\% \pm 0.3\%$ of HFSCs CD200+ (from the bulge)	2017	[16]
		(Rigenera®)			2. Yes*, improvement of $29\% \pm 5\%$ for the treated area after 23 weeks ($< 1\%$ for the placebo area)		
			24 (24) 24M, age range 19-63 years, with AGA	1. Hair density after ANA-	1. Yes, improvement of 65 ± 5 hair/cm ² in the		
			First group: 18M	PRP 2. Hair density after AA-	ANA-PRP treated area and no significant changes in placebo area after 12 weeks ($p < 0.05$)		
Gentile, et al.,	Randomized Controlled	HFSCs (ANA-PRP)	treated with ANA-PRP Second group: 6M (3	PRP 3. Growth factor	2. Improvement of 90 ± 6 hair/cm ² and	2017	[17]
	Blinded Half-head	and AA-PRP)	treated with AA-PRP prepared with Arthrex Angel System, 3 with AA-PRP produced using Regen Cell Therapy) Another population of 5 patients (3M, 2F,	quantification (hair regrowth) after ANA-PRP 4. Growth factor quantification (hair regrowth) after AA-PRP in Arthrex and Regen 5. Growth factor comparison after AA-PRP or ANA-PRP	decrease of 73 ± 24 in the Arthrex and Regen AA-PRP treated areas, respectively after 6 months ($p < 0.05$) 3. No, $p > 0.05$ 4. No, $p > 0.05$ 5. Yes, but the improvement was only statistically significant for VEGF using the Regen collection system ($p = 0.0283$)		
			age range 20-60 years,				
			with no apparent hair loss) was selected for				

			analyzing growth factor				
		HFSCs	78 (78) 56M, 22F, all patients with AGA	1. Hair density after PRP	1. Yes*, improvement of 65 ± 5 hairs/cm ² (31 $\pm 2\%$) for the treated area and $< 1\%$ for the placebo area 12 weeks later the last		
Gentile, et al.,	Controlled Half-head	(Micrografts containing	First group: 21 treated	treatment 2. Hair density after	treatment 2. Yes*, improvement of 39 ± 5 hairs/cm ² (30	2019	[18]
		HF-MSCs and PRP)	with HF-MSCs injections Second group: 57 treated with ANA-PRP	Micrografts treatment	$\pm 5.0\%$) for the treated area and $< 1\%$ for the placebo area 12 weeks later the last treatment		
				1. Hair density after HD-	1. Yes*, improvement of $33 \pm 7.5\%$ and $27\% \pm 3.5\%$ (23 and 44 weeks after the last treatment, respectively) for the treated area		
	Randomized	HFSCs	35 (33)	AFSCs micrografts in	and $< 1\%$ for the placebo		
Gentile, et al.,	Controlled Blinded	(Micrografts containing	23M, 12F, age range 21-70 years, all of them	treated and placebo areas 2. Immunophenotypic	2. Yes*, HF-MSCs CD44+ (from DP) increased $6.1\% \pm 0.2\%$ and HF-ESCs	2019	[19]
	Half-head	HD-AFSCs)	with AGA	Identification and Cell Counting 3. Number of HFs after treatment	CD200+ (from bulge) increased $2.9\% \pm 0.8\%$ 3. Yes, improvement of 1.4 ± 0.27 HFs per mm ² when compared to baseline values (0.46 ± 0.15) 44 weeks after the last treatment ($p < 0.05$)		
M, male; F, female; AGA, Androgenetic Alopecia; ADRC, Enriched Autologous Fat; ADSC, Adipose-derived Stem Cell; ADSC-CM, Adipose-derived Stem Cell in Conditional Medium; BMNC, bone marrow-derived mononuclear cells; SVF, Stromal Vascular Fraction; AFSC, Autologous Follicular Stem Cell; HFSC, Hair Follicle Stem Cell; PRP, Platelet Rich Plasma; ANA- PRP, Autologous Non-activated Platelet Rich Plasma; AA-PRP, Activated Platelet Rich Plasma, HD-AFSC, Human Intra and Extra Dermal Adipose Tissue-derived Hair Follicle Stem Cells.*p value not reported. Adapted from Gentile and Garcovich, 2020 [20].							

Table 1: Results of some of the included studies.

The Normal Hair Follicle Cycle

The normal HF consists of a mini-organ composed of keratinocytes, Dermal Papilla (DP), matrix, root sheath and bulge [21]. The tissue repair - hair regeneration from telogen to anagen phase - depends on the activation of Hair Follicle Stem Cells (HFSC), which are regulated by a series of other cells that compose the HFSC niche, including adipose tissue, blood vessels, nerves and immune cells [21-24]. The integration between all these components induces HFSCs to begin a regenerative cycle or to remain quiescent, based on activating or inhibitory signals expressed by a group of niche cells [21].

The DP plays a substantial role in the HF cycle by creating another niche containing mesenchymal cells, which boost the proper hair shaft elongation and the maintenance of hair growth. The interaction between HFSCs and DP cells is based on the ability of the DP to secrete growth factors and cytokines, a decrease of anagen factors [Insulin-Like Growth Factor 1 (IGF-1), Vascular

Endothelial Growth Factor (VEGF) and basic Fibroblast Growth Factor (bFGF)] leads to the entrance of the HF in the catagen phase, whereas an increase of cytokines [interleukin 1 alpha (IL- 1- α), Tumor Necrosis Factor beta (TNF- α) and Transforming Growth Factor beta 1(TGF- β 1)] have inhibitory and pro-apoptotic effects [1,22]. This means that interfering in these pathways' activation/inactivation balance could control HFSC activity, stimulating or inhibiting the anagen entry. That said, in diseased states, there is an invasion of cells that were not a part of HFSC's niche, therefore inducing dysregulated hair growth [21].

Changes in Hair Follicle Cycle: Impacts of Androgenetic Alopecia in Hair Loss

Despite there is no assurance of what exact genes are involved in hair loss, some of them can be highlighted, such as Epidermal Growth Factor (EGF), Fibroblast Growth Factor (FGF), Lymphoid-Enhancer Factor 1 (LEF-1), desmoglein, activin and others [25]. Regarding AGA, a study by Karnik et al. [26] analyzed scalp biopsies using microarray and concluded that a series of genes related to HF development (BARX2, EGFR, INHBA, MSX2, OVOL1, KRTs, KRTAPs, RUNX3 and TIMP3) were under expressed in patients with the disease [26].

In genetically susceptible individuals, the persistent stimulus of androgen receptors of HF in AGA results in a misbalance in the expression of signaling factors genes, changing the hair cycle dynamics; while the duration of the anagen phase decreases, the telogen phase increases, which results in a progressive miniaturization and vellus transformation of the terminal HF and possible baldness as a final outcome [1,2,27].

Nevertheless, unlike scarring alopecia, which provokes irreversible hair loss due to bulge HFSC's invasion, non-scarring alopecia like AGA preserves HFSC, conferring the possibility of a reversible hair loss prognosis [2,28]. Thus, this reversible condition creates a variety of possibilities for new AGA treatment modalities with the goal to revert hair loss and promote hair growth [29].

Management of Androgenetic Alopecia: Conventional Treatments and the Advent of New Therapies Based on the Use of Stem Cells

Conventional treatments for AGA, such as minoxidil, finasteride and dutasteride, may fail to treat all patients thoroughly [15]. In this context, SCs based therapies and their possibility to interfere with HFSC reactivation, hair cycle and HF regeneration have gained popularity [29]. Knowing that adipose tissue, bone marrow, umbilical cord, HF and other tissues can be a source of stem cells that can be used for the repair or regeneration of many body tissues (including hair), some alternative therapies for AGA based on SCs have been proposed [15,28,29].

Adipose-Derived Stem Cells (ADSCS)

The adipose tissue contains a variety of cells, such as adipocytes, endothelial cells, fibroblasts, leukocytes, mural cells and adipose-derived stem cells (ADSCs) [30]. ADSCs can be obtained by a little invasive liposuction from different parts of the human body [31] and are able to differentiate into mesenchymal lineage cells, stimulate HFs and promote hair growth [8,14,30,31]. Considering adipose tissue as a source of SCs, a study by Zanzoterra et al. analyzed the outcomes of the application of ADSCs and growth factors in 3 patients submitted to hair restoration surgery [32]. During the procedure, the researchers isolated the remaining tissues of the patient's scalp (hypodermis and adipose tissue), that normally are discarded and processed them using Rigenera®. The obtained suspension was injected into the patient's scalp and the analysis showed that the Rigenera®'s collected cells were young and were in their active cycle phase, contributing to hair growth, engraftment of the transplanted hair and helping tissue regeneration and healing of the micro-wounds [32]. The Rigenera System® involves the use of a special medical device called Rigeneracons (with a stainless-steel grid with 100 hexagonal holes of 50 μ each surrounded by six microblades) that mechanically fragments pieces of scalp skin tissue containing hair follicles and stem cells from the patient-augmented by 1 mL of saline solution. The processed tissue samples produce a suspension containing micrografts, growth factors and stem cells. This suspension is then injected or applied to areas of the scalp where hair growth is desired, such as in cases of male or female pattern baldness. The goal of this technique is to promote hair growth and stimulate the regeneration of hair follicles in areas affected by alopecia [32].

Another technique used in hair transplantation is the Regen Blood Cell Therapy or Arthrex Angel system, a technique that involves the use of Platelet-Rich Plasma (PRP) with growth factors and other bioactive proteins that can promote hair follicle regeneration. During the hair transplant procedure, PRP obtained from the Arthrex Angel system is injected into the scalp in areas where hair growth is desired [17].

Like the Arthrex Angel System, Regen Cell Therapy is a hair transplant technique that involves the use of regenerative cells to stimulate hair growth. This therapy uses the patient's own stem cells and growth factors found in their blood or fat tissue. These regenerative cells are processed and then injected into the scalp to promote hair follicle regeneration. The procedure typically begins with a small sample of the patient's blood or fat tissue being collected. The sample is then processed to isolate regenerative cells, such as Platelet-Rich Plasma (PRP) or mesenchymal stem cells. These concentrated regenerative cells are then injected into areas of the scalp where hair growth is desired. Regen Cell Therapy aims to stimulate the formation of new hair follicles, improve the health of existing follicles and promote overall hair growth. The technique is intended to be a natural and minimally invasive approach to treating alopecia [17].

Within the same context, a study by Fukuoka and Suga used Adipose-derived stem cell in conditional medium (ADSC-CM) in 21 patients with alopecia [8]. At the end of the treatment, it was observed new hair growth and also an increase in hair count; with these results, they concluded that the ADSC-CM treatment was efficient, safe, little invasive and did not require special equipment to perform [8]. These positive results using ADSC-CM were shared by Lee, et al., in a study with 30 patients with AGA and also by Narita, et al., in a study with 40 patients with the disease [9,33].

Another source of SCs is the Stromal Vascular Fraction (SVF), a mixed population of stem/stromal cells isolated from adipose tissue [30]. A study by Öztürk and Bekerecioğlu investigated the effectiveness of SVF treatment in 20 AGA patients [10]. After 3 months, an increase in both hair density and thickness was detected, showing that the SVF was safe and effective as an alternative for patients with AGA [10]. Similar findings were described by Kuka, et al., in a study with 71 patients with AGA using adipose-derived regenerative cell (ADRC)-enriched autologous fat grafts [11].

Additionally, Stevens, et al., investigated the use of SVF in 10 patients with AGA combining this technique with PRP in a method called Platelet-Rich Stroma (PRS) [34]. At the end of the study, hair density significantly increased without any side effects [34]. A similar study by Butt, et al., also reported the efficacy of SVF combined with PRP (hair density increased by 51.6% in patients treated with SVF-PRP versus 21.5% in those who only got PRP) [12].

Another study, this time using Adipose-Derived Stem Cell Constituent Extract (ADSC-CE), evaluated the use of ADSCs in 38 patients with AGA. The researchers found a significant increase in hair count in the intervention group (treated with a topical solution of ADSC-CE) when compared to the control and this increase was also verified when hair diameter was analyzed [13]. These findings are in agreement with the authors mentioned above so the use of ADSCs shows a potential therapeutic strategy for hair growth in patients with AGA.

Bone Marrow-Derived Stem Cells (BMSCs)

Elmaadani, et al., analyzed the safety and efficacy of the injection of autologous Bone Marrow-Derived Mononuclear Cells (BMMCs) (including SCs) [15]. In this study, 20 patients with resistant AGA received an injection containing autologous SCs and after 6 months there was a significant improvement in hair width and density, with BMSC therapy being more effective in female AGA patients when compared to male patients (which was also observed in a study using ADSCs) [14,]. Although bone marrow is the most important source of SCs in the human body, there is a lack of studies including BMSCs in AGA, mainly due to the difficulty of accessing and obtaining these kinds of cells [10].

Umbilical Cord-Derived Stem Cells (UCDSCs)

Human Umbilical Cord Blood-Derived Mesenchymal Stem cells (hUCB-MSC) and their secretory factors have been suggested to have a therapeutic role in tissue regeneration, therefore been used in some neurological diseases, bone and cartilage defects and skin damage [35-38]. Considering that a study by Bu, et al., was able to demonstrate the differentiation of hUCB-MSC in HF cells, which was also proposed by Bak, et al., in a study with mice. Both findings suggest that hUCB-MSC could be an alternative therapeutic treatment for AGA, primarily due to the fact that it is non-invasive, has an easier collection and also is more efficient and abundant than the methods that work with BMSCs or ADSCs [38-41].

Hair Follicle-Stem Cells

The hair follicle contains an enormous niche for adult SCs: the bulge region, which shelters melanocytic and epithelial stem cells, hair follicle structures and sebaceous glands [42]. With that concept in mind, a study by Yu, et al., was capable of identifying a

new group of cells with the capacity to differentiate into neurons, smooth muscle cells and melanocytes by culturing human hair follicle- derived cells in human Embryonic Stem Cell (hESC) medium [42]. They found that this isolated group of cells, termed by them as human Hair Follicle Stem Cells (hHFSC), was located in the bulge area of the HF just like epithelial and melanocytic SCs; however, unlike these cells, HFSCs did not express any lineage-specific markers, which gives them multipotent and promising plasticity properties [42]. After the discovery by Yu, et al., the use of HFSCs, the “new” component of HF’s bulge, started to gain notoriety as an alternative treatment for multiple conditions, mainly due to their capacity of being generated from autologous adult tissues and to the fact that human scalp tissues are easily accessible. HFSCs are multipotent cells that have a significant self-renewal ability and a slow cell cycle with proliferative capability [21,23,42,43]. Considering their potential to differentiate into many types of cells, new therapies based on the reactivation of HFSC have been proposed to treat and reverse hair loss (including AGA) by enhancing hair follicle growth and regeneration [2,2229].

In this context, for the first time, a study used an injection of HFSCs preparations on AGA, obtaining a positive therapeutic effect without major side effects [16]. Gentile, et al., developed a new method to isolate HFSCs using Rigenera®, the same methodology used by Zanzottera, et al., in a study with ADSC, to provide autologous micro-grafts enriched of hHFSCs to patients with AGA [1,32]. The cell suspension was injected into the scalp of 11 patients affected by AGA and besides an increase in hair density of the treated area, both Hair Follicle-Derived Mesenchymal Stem Cells (HF-MSCs) CD44+ (from DP) and Hair Follicle Epithelial Stem Cells (HF-ESCs) CD200+ (from bulge) count were improved after the treatment. Considering the identification and counting of HFSCs and the clinical safety and feasibility in HFSCs-treated scalp, Gentile, et al., concluded that the methodology using HFSC isolated from the patients themselves was promising for the AGA treatment [16,44].

In the same year of this cited study (2017), Gentile, et al., tested a PRP methodology as a potential alternative for AGA using two different protocols: autologous non-activated PRP (ANA-PRP) and autologous activated PRP (AA- PRP) [17]. Average 6 months after the last treatment infiltration, both hair count and density were higher in the ANA-PRP group when compared to the AA-PRP group. After all these results, the group concluded that PRP could also be a valid alternative for AGA treatment [17].

Two years later, Gentile, et al., proposed a series of studies about hair growth in AGA patients [18,19]. The first one, published in April 2019, reported the clinical effectiveness of HF-MSCs and PRP treatments [18]. In this work, 21 patients received injections containing autologous micrografts of HF-MSCs prepared using Rigenera®, while 57 patients were treated with ANA-PRP. Considering micrografts, the results after 12 weeks demonstrated that hair count and density increased over baseline values. For PRP, the results showed that both hair count and density also increased, outcomes that had already been demonstrated by Gentile, et al., in their previous work. Therefore, the authors concluded that both micrograft-HF-MSCs and PRP infusions represented an effective and safe procedure alternative for patients with AGA [17,18].

The second study [19], published in July 2019, evaluated the hair growth results after the use of a new autologous technique, which they called “human intra and extra dermal adipose tissue-derived hair follicle stem cells” (HD-AFSCs). In this study, HD-AFSCs were considered the cellular population containing HF-MSCs and HF-ESCs. The group of researchers analyzed the results obtained by their past work aforementioned, performed centrifugation of scalp tissue by the Rigenera® procedure and created what they called the “Gentile procedure”, which adds a splitting phase before the centrifugation [17]. Three injections each 45 days were applied in 33 patients with AGA. After the last injection, Gentile, et al., found that HF- MSCs CD44+ (from DP) increased $6.1\% \pm 0.2\%$ and HF-ESCs CD200+ (from bulge) increased $2.9\% \pm 0.8\%$ [17]. In their previous work used Rigenera® procedure, these increased numbers were, respectively, $5\% \pm 0.7\%$ and $2.6\% \pm 0.3\%$ [13]. These results demonstrated that the “Gentile procedure”, when compared with their 2017’s previous work, showed better outcomes in hair growth. In other words, the use of micrografts containing HD-AFSCs may be a valid, safe and potential treatment alternative for patients with AGA [19]. Another controlled randomized study by Gentile, et al., published in January 2020 compared hair regrowth with micrografts containing HF-MSCs versus placebo in 27 patients with AGA [20]. Autologous micrografts were prepared using the “Gentile protocol” and were applied in targeted areas, while the control areas were injected with a placebo. At the end of the study, all patients displayed an increase in both hair count and density compared with the baseline in the targeted area, while the control area showed a decrease ($p < 0.0001$). It was also performed an immunophenotypic cell identification of all samples and the authors found CD44+ HF-MSCs and CD200+ HF-ESCs [20]. Therefore, Gentile, et al., succeeded in collecting HFSCs with minimal manipulation based on the centrifugation of the scalp’s fragments obtained by punch biopsy, which could be fundamental for treatment alternatives against hair loss [44].

Due to the non-existence of standardized preparation techniques for the use of Autologous Stem Cell-Based Therapy (ASC-BT) and PRP, choosing the best protocol for each method might be a challenge [44]. In order to prevent future studies from having different outcomes from the same methods, which have already been registered in Gentile et al. previous studies, a recent work by Gentile, et al., presented a suggested guideline based on the most recent European rules and scientific data published for the use of ASC-BT and PRP techniques in hair regrowth [17,44]. Thus, regarding clinical use and related indications of each method, the group highlighted that (a) ASC-BT can be indicated for AGA treatment if the procedure involves a functional use and is in agreement with the European laws: cellular products captured in a one-step procedure via minimal manipulation; (b) PRP techniques can be used for AGA treatment in selected patients: grade I-IV for males (Norwood-Hamilton scale) and grade I-II for females (Ludwig scales). That said, Gentile, et al., concluded that although there is a scarcity of standardized protocols, the data from several studies showed positive outcomes coming from both ASC-BT and PRP methods, which suggests that they are a safe and effective treatment and therefore, should be considered as an alternative therapy for hair regrowth in AGA patients [44].

Studies Limitations

Noteworthy limitations include the challenge of culturing cells in numbers adequate for human use, the need to conduct this expansion in Good Manufacturing Practice (GMP) research centers and the viability of the extended cells [18,45]. The low number of patients [10]. Despite some limitations of the study (small sample size, poor follow-up data, nonblinded analysis), this study showed that stromal vascular fraction with fat injection is a safe and promising alternative approach to treating hair loss in men and women and served as the precursor to the phase II investigation described in this report [11].

The study by Tak, et al., has some limitations [13]. First, the study duration was 16 weeks, which is a relatively short period for a clinical trial. Therefore, the safety data may not be sufficient to conclude the long-term effect of ADSC-CE application. Second, although they estimated the total number of participants needed for this trial based on a published study, the enrolled number of female participants was considered small to verify the efficacy and safety of ADSC-CE, especially in female- pattern hair loss. Third, the results may not be generalizable because the data are from a single center. In addition, they were unable to measure the type and concentration of growth factors in ADSC-CE. Further studies on the mechanism of action of growth factors in the hair cycle will help to understand the efficacy and safety of ADSC-CE in the treatment of AGA. Finally, the study failed to demonstrate histologically the amount of trial product that reached the hair follicles.

Limitation in dermal-inductive tissue grafting due to the fact that it was not possible to generate more hair follicles than those obtained from donor tissues. To overcome this limitation, different approaches and experimental models using fresh isolated or cultured cells of dermal and dermal/epidermal origin were tested. Most of them involved neonatal and embryonic murine cells [17].

In the study by Avci, et al., 1 year after irradiation with Low-Level Laser (Light) Therapy, all lesions disappeared; hair density, length and diameter of hair shafts were the same in both irradiated and non-irradiated lesions; suggesting that LLLT only accelerates the hair regrowth process in AA patients [25]. However, the method for evaluating hair regrowth, density and thickness was not clearly stated, which was one of the main limitations of this study.

According to Egger, et al., stem cell-derived conditioned medium demonstrates potential as a future hair regrowth therapy; however, like any treatment modality, it has certain limitations [29]. In particular, the type and level of factors present in stem cell-derived conditioned medium can be highly variable and standardization of its preparation will be of utmost importance to improve its clinical use and outcomes. Furthermore, the rapid turnover and depletion of factors from stem cell-derived conditioned medium *in-vivo* may require large quantities and frequent application.

Although stem cell transplantation and stem cell-derived conditioned medium have demonstrated preclinical and some clinical success, each has its limitations that will need to be overcome. Stem cell transplantation is an expensive procedure and also raises concerns regarding tumorigenicity. Although stem cells may be more affordable and safer in terms of tumor development, both have some drawbacks [29]. While the results are certainly promising, larger and more robust double-blind controlled clinical trials are needed to further evaluate the exact mechanisms, therapeutic potential and safety of stem cell-based approaches to hair loss management [29].

Conclusion

With stem cells gaining notoriety, several techniques have been proposed to restore hair growth. In this review, we discussed the hair cycle and how some diseases, particularly AGA, can disturb its homeostasis. Additionally, we assessed some of the existing alternatives for AGA treatment in patients who do not respond to first-line therapy, drawing attention to the use of SCs to promote hair regrowth. Although there is still limited data to fully validate SC therapies, some techniques should be considered promising alternatives for AGA patients due to their efficiency and multipotent and plasticity properties. Among them, we highlight the isolation of HFSCs using Rigenera®, a technology that not only has shown great results in increasing hair count and density in patients affected by the disease but also is increasingly being imported to different countries around the world. Due to the popularity of hair transplantation to promote hair regrowth, another possible alternative for AGA patients is a method that uses HFSCs concomitantly with the performance of this transplant procedure, which could bring even more promising results allied to continuous treatment. Despite all the benefits of HFSCs for the AGA prognosis, further studies are still needed to validate and standardize the ideal conditions of each technique and guarantee the safe implementation of these methods.

Conflict of Interest

The authors declare they do not have conflicts of interest.

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