Regenerative medicine strategies for hair growth and regeneration: A narrative review of literature

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A B S T R A C T

Hair loss, or alopecia, is associated with several psychosocial and medical comorbidities, and it remains an economic burden to individuals and the society. Alopecia is attributable to varied mechanisms and features a multifactorial predisposition, and the available conventional medical interventions have several limitations. Thus, several therapeutic strategies for alopecia in regenerative medicine are currently being explored, with increasing evidence suggesting that mesenchymal stem cell (MSC) implantation, MSC-derived secretome treatment, and blood-derived platelet-rich plasma therapies are potential treatment options. In this review, we searched the Cochrane Library, MEDLINE (PubMed), EMBASE, and Scopus using various combinations of terms, such as “stem cell,” “alopecia,” “hair loss,” “Androgenetic alopecia,” “male-pattern hair loss,” “female-pattern hair loss,” “regenerative hair growth,” “cell therapy,” “mesenchymal stem cells,” “MSC-derived extracellular vesicles,” “MSC-derived exosomes,” and “platelet-rich plasma” and summarized the most promising regenerative treatments for alopecia. Moreover, further opportunities of improving efficacy and innovative strategies for promoting clinical application were discussed.

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1. Background

Hair is found on various parts of the human body, and it performs various critical functions, such as body protection, heat insulation, camouflage, sebaceous secretion, sensory perception, and social interactions [1]. The hair follicle (HF) grows following a cycle of dynamic and complex processes, which mainly alternate in three phases: rapid growth (anagen), regression (catagen), and quiescence (telogen) [2]. A mature human HF is a complex structure comprising multilayered, concentric epithelial basement cylinders of keratinocytes and distinctive mesenchyme of dermal papilla cells (DPCs) as the main components [3]. Human DPCs (hDPCs) originate from B lymphocyte-induced maturation protein-1 † fibroblasts, also known as dermal stem cells (DSCs), during embryonic development. DSCs, which can stimulate epidermal hair follicle stem cells (HFSCs), are widely studied as key controllers of the HF growth cycle throughout an animal’s life cycle [4,5]. Multiple factors within and outside HFs can influence growth [6]. Although HFs are protected and maintained through their association with immune responses against pathogens and different tissue regeneration and healing processes, hair abnormalities or loss (alopecia) commonly occurs in both males and females of all ages, affecting quality of life, attractiveness, and self-esteem. Reportedly, alopecia can lead to psychiatric disorders and increased risks of diseases, such as myocardial infarction and metabolic syndrome [5,7,8].

Alopecia is broadly categorized into two subtypes: scarring or cicatricial alopecia (CA) and non-scarring or non-cicatricial alopecia (non-CA) [9]. CA destroys HFs with or without scar formation [10]. Reportedly, CA comprises approximately 5% of all cases of alopecia and describes multiple subtypes of hair loss caused by unknown inflammatory mechanisms. CA is further subdivided according to the inflammatory response as primary neutrophilic, primary lymphocytic, and mixed subtypes [10,11]. Neutrophilic CA includes folliculitis decalvans and dissecatingcellulitis of the scalp; lymphocytic CA comprises central centrifugal cicatricial alopecia, discoid lupus erythematosus, lichen planaralis, frontal fibrosing alopecia, Graham–Little syndrome, pseudopelade of Brocq, follicular mucinosis, and keratosis follicularis spinulosa decalvans; and mixed CA includes acne keloidalis, acne necrotica, and erosive pustular dermatosis of the scalp. Non-CA is often a clinical feature of various diseases, either through direct HF destruction or indirectly via HF dysfunction. Disturbances of HF function lead to excessive terminal hair loss, follicular miniaturization, and progressive hair thinning. Non-CA includes several different types summarized in Table 1, with the exception of the two most common and widely explored, androgenetic alopecia (AGA) and alopecia areata (AA), which are described in detail as follows:

AGA is the most common cause of hair loss, affecting 30–50% of men (male-pattern hair loss [MPHL]) and approximately 30% of middle-aged women (female-pattern hair loss [FPHL]) [22]. The mechanisms of AGA are multiple, interlinked, and common to both MPHL and FPHL. Among them is the hypothesis of oxidative stress (OS) resulting from increased expression of pro-inflammatory cytokines attributable to chronic perifollicular microinflammation. It is suggested that in the genetically predisposed, OS works in tandem with high androgen levels and various environmental factors to interrupt the corticotropin-releasing hormone pathway and cortisol levels that lead to AGA [23]. Moreover, gene variants in the predisposed are suggested to play a critical role in the etiology of AGA by increasing the activity of 5α-reductase or the sensitivity of androgen receptors [24]. AGA is characterized by HF miniaturization caused by perturbation of the growth cycle via dihydrotestosterone (DHT) accumulation. DHT accumulates because of the inhibition of testosterone metabolism by 5α-reductase [25,26]. AGA is synonymous with the histological observation of lymphocytes and mast cells around the miniaturized HFs and bulge area and reduced numbers of proliferating progenitor cells amidst an intact quantity of HFSCs [27]. Perifollicular inflammatory infiltration and the involvement of inflammatory genes encoding caspase-7 and tumor necrosis factor (TNF) provided proof of the inflammatory hypothesis in AGA [23]. In addition, exposure to high OS levels in the skin leads to the accumulation of reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, in HFs, which overcomes the follicular antioxidant defense capacity and leads to premature and dysfunctional hDPCs [28]. Without intervention and with exacerbation, AGA becomes irreversible.

AA is the second most common form of non-CA, and it affects both sexes equally, with a prevalence of 0.1%–0.2% in the general population and no significant racial preponderance [29]. The etiopathogenesis of AA remains elusive. However, it has been demonstrated that AA is associated with systemic autoimmune activation in isolation as an acquired autoimmune disorder (AD) or as a...
Table 1

| Type                                | Description                                                                 | Causative factors                                                                                                                                                                                                 |
|-------------------------------------|------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Senile or senescent alopecia        | • Aging leads to reduced hair density and thinner fibers leading to hair loss.| • The impact of aging dermal environment on HF is unclear.                                                                                                                                                    |
|                                     | • Shares various features with AGA.                                          | • However, evidently aging scalp shows striking structural and biological changes in HF environment that affect hair growth, and                                                                                 |
|                                     | • Begins later in life, (70 years old)                                       | • A phenomenon of gradual thinning of hair with age due to increasing number of HF switching from anagen, to telogen                                                                                                 |
|                                     | • Distinguished by the synchronization of HF miniaturization                 | • Direct damage to the mitotic/metabolic activity of the HF.                                                                                                                                                    |
|                                     | • Does not respond to 5α-reductase inhibitor treatment.                      | • Target the desmosomal proteins, which are overexpressed in the HF epithelium.                                                                                                                                 |
| Anagen effluvium (AE)               | • Characterized by the abrupt shedding of anagen hairs affected by an acute insult. | • Hair shedding may result from cleavage of the ORS.                                                                                                                                                                     |
|                                     | • Commonly observed as a result of anticancer treatment, heavy metal poisoning or radiotherapy. |                                                                                                                                                                                                                      |
|                                     | • Associated with AA, pemphigus vulgaris, local traumas and infections.       |                                                                                                                                                                                                                      |
|                                     | • Diagnosis is based on history and a positive pull test with dystrophic anagen hairs. |                                                                                                                                                                                                                      |
| Telogen effluvium (TE)              | • Terminology introduced by Kligman in 1961                                  | • A wide range of factors and diseases can induce hair loss through various mechanisms.                                                                                                                             |
|                                     | • Characterized by abrupt generalized shedding of telogen hairs.            | • Common induction factors include stress, nutritional deficiencies, bariatric surgeries, hormonal imbalances in pregnancy and menopause, thyroid dysfunction, diabetes, autoimmune diseases, polymyositis, Sjögren’s syndrome, febrile or infectious diseases, neoplastic diseases, chronic poisoning, certain drugs and chronic exposure to low-dose toxic agents |
| Alopecia secondary to SLE            | • Hair loss in SLE can be either diffuse non-CA, such as TE, AGA, AE, and lupus hair, or patchy non-CA, such as AA, the most common type of alopecia observed in SLE. | • Results from both severe catabolic and elevated levels of circulating pro-inflammatory cytokines in the hair growth cycle cycle                                                                                       |
| Trichotillomania (TTM)              | • Often coexists with skin picking disorder (SPD), characterized by repeated pulling out of hair resulting in hair loss | • Reward seeking and loss/harm avoidance play important roles in human behavior, and when there is dysfunction in reward processing, maladaptive behaviors, such as TTM and SPD may occur. |
| Traction alopecia (TA)              | • Majorly affects individuals who wear various forms of traumatic hairstyling for a prolonged period of time. | • Hair loss caused by prolonged or repetitive traction and tension on the hair.                                                                                                                                       |
|                                     | • Risk is increased by extent of pulling and duration of traction, and the use of chemical relaxation. |                                                                                                                                                                                                                      |
|                                     | • The frequent use of tight buns or ponytails, the attachment of weaves or hair extensions, and tight braids are believed to be the highest risk hairstyles. |                                                                                                                                                                                                                      |
|                                     | • TA can also occur in the setting of religious and occupational traumatic hairstyling. |                                                                                                                                                                                                                      |
|                                     | • Without appropriate intervention TA may progress into an irreversible CA if traumatic hairstyling persists. |                                                                                                                                                                                                                      |
|                                     | • TA is characterized by marginal alopecia and non-marginal patchy alopecia features and distinguished by its preservation of the frontal and/or temporal rim, dubbed the "fringe sign and detection of the ongoing traction by the presence of hair casts through a dermoscopy. |                                                                                                                                                                                                                      |
| Lipedematous scalp (LS) and lipedematous alopecia (LA) | • These rare alopecias that mostly affect middle-aged women with a thick subcutaneous layer of the scalp and soft and boggy scalps. | • Could result as a complication of the differentiation process of adipocytes.                                                                                                                                       |
|                                     | • Both LS and LA reportedly commonly coexist with each other.               |                                                                                                                                                                                                                      |

comorbidity with diseases such as systemic lupus erythematosus (SLE), sometimes referred to as non-CA in SLE [30,31]. SLE has been described as a rare, heterogeneous autoimmune, and autoinflammatory disease with complex etiopathology and clinical manifestations. SLE is characterized by the dysregulation of immune cells, copious amounts of pathogenic autoantibodies, and accumulated immune complexes [31,32]. Immune disorders to SLE include abnormal proliferation, differentiation, activation, and dysfunction of innate and adaptive, immune components such as natural killer (NK) cells, monocytes, macrophages and dendritic cells (DCs), B lymphocytes, and T lymphocytes respectively, which in combination with continuous inflammation culminate in multiple tissue and organ damages. Therefore, patients with AA or non-CA in SLE present significantly dysregulated serum levels of T helper (Th1) cytokines (e.g., interleukin [IL]-1β, IL-2, IL-12, TNF-α, interferon-γ), Th2 cytokines (e.g., IL-4, IL-10, IL-13, IL-25, IL-31), regulatory T cells (Treg), and Th17 cytokines (e.g., IL-17A). Similarly, as observed in AGA, OS caused by interruption of the balance between ROS production and antioxidant activity probably contributes to the pathogenesis of AA [33]. The prognosis of AA is unpredictable, and the disease has variable clinical manifestations, often appearing in patches and sometimes arising in a more extensive distribution pattern [34]. In addition, reports indicate an initial regrowth of white hairs and an association with vitiligo in AA that is proposed to be attributable to immune attack of the hair bulb that mainly comprises melanocytes [30]. Tosti et al. [35,36] described the histopathological distinguishing feature of AA as lesions with peribulbar lymphocytic infiltration comprising cluster of differentiation (CD)8+ T cells in the follicular epithelium and CD4+ T cells around HFs. There are three types of AA based on extent of involvement: patchy AA, alopecia totalis, and alopecia universalis [37]. In addition, based on the pattern of involvement, AA can be of
the reticular, ophiasis, or sisaipho type, as well as a new variant described as acute and diffuse total alopecia, which is mainly observed in females. Other unusual patterns are perinevoid alopecia and linear AA [38].

Therapeutic products for alopecia have been extensively studied for many decades because of the continuous high demand by society for effective interventions to reduce the associated rates of mental and physical health disorders and the economic burden [39]. However, only a few pharmacological treatment options have been approved for clinical use by regulatory bodies, such as the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Moreover, these conventional therapies which mainly include corticosteroids, minoxidil, and 5α-reductase inhibitors such as finasteride and dutasteride, face significant challenges [40,41].

The pharmacological treatments are mostly tailored to specific types of alopecia. For instance, in CA, treatment majorly aims to stop inflammation using topical or intralesional corticosteroids, antimalarias, immunosuppressants, and anti-microbials. Several other potential remedies for intractable alopecia are under investigation, albeit with limited efficacy [42], and non-CA treatment mostly utilizes corticosteroids, minoxidil, and the 5α-reductase inhibitors such as finasteride and dutasteride, and depends on the type of alopecia as well. AGA being progressive with a risk of irreversibility, treatment is challenged by multiple factors including early initiation before the occurrence of overt HF miniaturization and management of the disease that must aim at identifying and eliminating exacerbating factors. The treatment of AA is commonly based on professional assessment with the Severity of Alopecia Tool (SALT), and to a great extent remains contestable with the multiple associated recurrences negating long-term outcomes of the conventional approaches [43]. AA treatment aiming to stop the immune episodes is sometimes effective in reversing the disease. However, it is suggested that hair regeneration in AA requires more than immunosuppression to restore normal HF function following the stoppage of the immune response [43].

Corticosteroids are associated with adverse events including hyperglycemia, blood pressure alterations and edema, hypothyroidism—pituitary—adrenal axis suppression, osteoporosis, immunosuppression, muscle wasting, and physical appearance changes such as moon facies, buffalo hump, and central trunc obesity [44]. Minoxidil is associated with adverse reactions in approximately 20% of patients, which include facial hypertrichosis observed in approximately 80% of patients during treatment, hypotension, acute pulmonary edema, and pulmonary hypertension. Contact dermatitis occurs probably because of propylene glycol, a common ingredient in topical minoxidil preparations, and other side effects include dizziness, sodium, and fluid retention, reflex tachycardia, headaches and, less commonly, electrocardiogram changes, paracardial effusion, and congestive heart failure [45,46]. Moreover, termination of minoxidil treatment results in progressive hair loss within a short period (12–24 weeks) [47]. Similarly, the 5α-reductase inhibitors are associated with undesirable events including a collection of sexual dysfunction effects such as decreased libido, erectile dysfunction, and reduced ejaculate volume, which are observed in approximately 2.1%–3.8% of patients, as well as mood disturbance and gynecomastia [48]. In addition, the drugs are potentially teratogenic, which is critically an irreversible event. Meanwhile, the drugs are mostly effective in younger adults, such as women younger than 50 years and men younger than 40 years. Taken together, the conventional treatment approaches for alopecia are contestable, and present significant challenges, which are prohibitive to regular clinical use. Thus, deciphering the pathogenesis and exploring the biology of hair toward identifying novel therapies and/or improving the effectiveness of existing interventions remains paramount. Recently, evidence-based regenerative medicine reports found that the application of various related therapeutic approaches is safe and effective in restoring the normal function of diseased tissues or organs including HF’s [27,49]. The approaches include stem cell (SC) therapy and blood-derived cellular therapies such as platelet-rich plasma (PRP) therapy for hair growth and regeneration. This review highlights current information on different regenerative strategies and discusses innovative clinical application plans for improving safety and effectiveness in the future.

2. Methodology

2.1. Literature search

The literature search mainly focused on original English-language articles on regenerative medicine and skin aging treatment approaches. Non-English articles were evaluated for pertinence. Databases including Medline, Embase, and Web of Science were searched using various combinations of the following search terms: “stem cell,” “alopecia,” “hair loss,” “AGA,” “MPHL,” “FFHL,” “regenerative hair growth,” “therapy,” “mesenchymal stem cells,” and “platelet-rich plasma.” A web search was conducted between March 2022 and June 2022 to investigate the clinical trial implementation status of cell therapy for hair growth and regeneration. The following websites were surveyed: ClinicalTrials.gov (https://clinicaltrials.gov/), a list of submitted regenerative medicine provision plans (Ministry of Health, Labour and Welfare, https://saiseiiryo.mhlw.go.jp/published_plan/index/1/2 and https://saiseiiryo.mhlw.go.jp/published_plan/index/1/3), and JRCT (https://jRCT.niph.go.jp/search).

3. Regenerative medicine-based treatment strategies

Regenerative medicine aims to restore or establish normal body function by replacing or regenerating cells, tissues, and organs. The replacement or regeneration process may utilize SCs, soluble or trophic molecules such as SC- or hematopoietic tissue-derived cytokines and growth factors, and gene-based therapies, as well as tissue engineering and reprogramming [49–51]. The reported current regenerative medicine-based therapies with promise in the treatment of alopecia include SC and PRP therapies [52,53]. The molecular mechanisms underlying the action of regenerative therapies remain elusive. SCs possess characteristic properties such as self-renewal, migration, anti-inflammatory, and immunomodulation functions, essential for the repair and restoration of injured tissues or organs [49]. The therapeutic effects of SCs were initially believed to be based on their abilities to migrate into damaged tissue (homing) and subsequently differentiate to replace the damaged tissues or organs. However, Gneccchi et al. [54] indicated that the therapeutic impact of SCs on diseased tissue occurs, at least in part, through the release of trophic (paracrine) factors. PRP exploits hematopoietic effects through the release of cytokines and various growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), and fibroblast growth factor (FGF), and it plays important roles in regenerating or restoration of tissues such as hair [53]. The use of PRP in the treatment of alopecia will be reviewed in detail in this article. Although still in infancy, several preclinical studies indicated that SC-based therapies for different pathologies are generally safe and potentially promising in the treatment of alopecia [52,53]. Nevertheless, given their recent inception compared to widely
studied conventional pharmacological treatments for alopecia, bottlenecks to the clinical application of SC-based therapies likely exist. The genuses of these bottlenecks are multifactorial, and they may include the origin tissue of MSCs, donor factors such as age, and challenges to large-scale cell production or cell manipulation. Such factors could lead to low SC transplant cell viability, poor homing and engraftment into injured tissues, SC heterogeneity, challenges related to optimum SC dose determination, and adverse events, such as graft-versus-host disease (GVHD) and increased risks of tumorigenicity [55]. However, there is increasing motivation in research and clinical practice to explore the potential of SCs for enhancing HF growth and regeneration through several robust clinical investigations and advanced innovation and manufacturing technology in industry and academia to continually discover novel and/or improve the existing treatment applications [53]. Moreover, to ensure consistency regarding safety, efficiency, and effectiveness, regenerative therapies are strictly regulated in accordance with set guidelines and standards or principles. The International Society for Stem Cell Research (ISSCR) set global standards for stem cell research and clinical translation, stipulating guidelines for preclinical research, clinical translation, and practice, to emphasize the importance of high standards of cell processing, manufacturing, and good manufacturing practice (GMP) [56]. The ISSCR and other international standards such as the Declaration of Helsinki, which is the cornerstone document on human research ethics, provide public reassurance that the production or handling of SCs adheres to quality standards in addition to the approval processes set by the different national regulatory authorities, such as the Pharmaceuticals and Medical Devices Agency (PMDA) and the Ministry of Health, Labor and Welfare in Japan [57], the US FDA [58], the EMA and its Committee for Advanced Therapies [59], and the Australian Therapeutic Goods Administration [60].

3.1. SC-based therapies for alopecia

Currently, SC therapies majorly utilize adult tissue-specific derived SCs, such as mesenchymal stromal cells (MSCs), SC-derived chemokines or growth factors (secretome), and bio-materials with regenerative properties, such as Lipogems therapy, in addition to the existing SC niche (microenvironment), to provoke an impactful restoration process [51]. It is against this background that this review discusses SC-based therapies for alopecia, which can be autologous or allogeneic, through a classification based on the use of purely isolated SCs with manipulation (culturing) or with minimal manipulation (cell-based therapies) and the utilization of an SC-derived secretome or extracellular matrix (ECM) (conditioned medium [CM]) or extracellular vesicles (EVs) such as exosomes (cell-free therapies).

3.1.1. Cell-based therapies

The adult tissue sources of MSCs with multipotent regenerative potential include adipose tissue (AD-MSCs) [61], bone marrow (BM-MSCs) [62], HFs of non-affected areas such as HFSCs and hDPCs [63], and perinatal sources, such as the placenta and its fetal adnexa (this review mainly provides details of umbilical cord blood-derived MSCs [hUC-MSCs]) [64]. Regarding induced pluripotent stem cells (iPSCs), because of the associated increased risk of tumorigenicity, therapies envisage the use of iPSC-derived SCs with lower risk known as iPSC-derived mesenchymal stem cells (iPSC-MSCs) [65]. MSCs can regenerate HFs and other organs in the skin, such as the sebaceous glands, through several mechanisms including the reversal of pathological mechanisms, regeneration of HFs, and creation of new HFs with organoid technology [66]. Conversely, in AA or non-CA in SLE, MSCs show promise in alleviating the often severe and refractory SLE under appropriate conditions, and thus indirectly treating the alopecia [32]. Management of ADs such as SLE is quite challenging and is beyond the scope of this article. However, of note, SLE flares can essentially be abated by gaining immune homeostasis and improve hair growth [67]. MSCs have strong anti-inflammatory and modulatory effects on innate and adaptive immunity [31,32,68,69]. Indeed, although Traggiai et al. [70] raised a special concern regarding MSCs in the treatment of SLE by demonstrating improved B cell proliferation and differentiation following the culture of MSCs obtained from patients with SLE and healthy donors with B cells in vitro, increasing evidence suggests that MSC therapy could effectively treat SLE. In studies reported by Kamen et al. [71] and Liang et al. [72] MSC treatment was well tolerated and effective against the disease conditions. Wang et al. [73] also reported that MSC transplantation effectively treated SLE by increasing regulatory T cell (Treg) counts and decreasing Th17 counts in TGF-β- and PGE2-dependent manners. Allogeneic MSC transplantation was also well tolerated and resulted in long-term clinical remission in patients with SLE [74]. Furthermore, Liang et al. [72] observed that patients with refractory SLE who underwent MSC transplantation experienced clinical improvement without severe side effects. In another study by Barbado et al. [75], MSC transplantation exhibited low immunogenicity, improved proteinuria levels, reduced mediastinal masses, and improved renal function without clinical signs of acute immune rejection.

Notwithstanding, the molecular mechanisms underlying the MSC therapeutic effects in SLE are not yet completely deciphered [69]. However, potency of the anti-inflammatory and immunomodulatory effects of the MSCs exerted through intercellular contact and paracrine pathways have been described and discussed [31,32,68,69]. Triggers of anti-inflammatory effects of MSCs are multifactorial including the inflammatory environment and its associated proinflammatory factors such as IL-6, TNF-stimulated gene 6, IGF1, human leukocyte antigen G (HLA-G), and PGE2. MSC immunosuppression is through the production of nitric oxide, Indoleamine-pyrrole 2,3-dioxgenase (IDO), PGE2, TGF-β, IL-6, and HLA-G protein [32]. MSC modulatory effects are dose and cell type dependent; i.e., high numbers of allogeneic MSCs are suppressive, whereas lower numbers could up-regulate the immune cell responses, and MSCs obtained from SLE patients (autologous MSCs) are reportedly dysfunctional [69,76]. Tang et al. [32] highlighted different published findings on the interactions between MSCs and immune cells. (i) MSCs secreted factors inhibit NK cell cytotoxicity and secretion of inflammatory cytokines, such as IFN-γ or TNF-α. IDO and PGE2 reportedly mediate the downregulation of activating NK receptors: natural cytotoxicity triggering receptor 3 (CD337 or Nkp30), natural cytotoxicity triggering receptor 2 (CD 336 or Nkp44), and NKG2D by MSCs in inhibiting NK cell proliferation and cytotoxicity. Additionally, TGF-β and IL-6 can reduce NK cell effector function and encourage NK cell differentiation. (ii) MSCs induce differentiation of pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages, which express macrophage mannose receptor, CD206, and haptoglobin—hemoglobin scavenger receptor, CD163, and secrete high levels of IL-10 and chemokine (C–C motif) ligand 18 that induce strong immunosuppressive effects. (iii) MSCs nourish the endurance of monocytes by inducing morphological and physiological transformation following phagocytosis of infected MSCs to mediate a subsequent immunomodulatory effect. (iv) MSCs modulate DC activation and tolerogenic characteristics and program neutrophils to develop an immunosuppressive phenotype. A study by Yuan et al. [69] demonstrated the possibility of allogeneic MSCs in suppressing inflammation in SLE by up-regulating tolerogenic CD1c⁺ DCs and the levels of serum cytokine Fms-related tyrosine kinase 3-ligand (FLT3L), a key regulator of DC commitment in hematopoiesis. FLT3L expression in
MSCs is induced by IFN-γ through JAK/STAT signaling pathway. (v) B cells exhibit hyperactivity in SLE and increased expression of especially toll-like receptors 7/9, which contribute to the inflammatory state [77]. MSCs inhibit the differentiation of B cells into plasma cells, reduce the production of immunoglobulin, and downregulate the expression of chemokine receptors, such as CXCR4, CXCR5, and CCR7 on the surface of B cells, which determines the response to the chemotactic ligands, stromal cell-derived factor 1, B lymphocyte chemoattractant and chemokine (C–C motif) ligand 19/21 respectively. In addition, although reduced monocyte chemoattractant protein 1 (MCP1) expression in SLE impairs B cell inhibition, the presence of T cells and cell—cell contacts between MSCs and T cells enhances B cell inhibition. It is known as well that MSCs stimulate IL-10-producing B regulatory cells under latent immunological conditions. (vi) MSCs inhibition of T cell proliferation and production is dependent on MCP1 and other MSC secreted factors, such as TGF-β1/2/3, and IL-10 via inhibiting cleavage of caspases [78]. MSCs decrease the ratio of Th1 to Th2 cells and increase the number of Treg cells to regulate the immune environment in SLE. The regulation of Th17 cells is complicated, however, MSCs down-regulate Th17 cells through the regulation of TGF-β and PGE2 in SLE patients [79].

3.1.1.1. Treatment of alopecia using AD-MSCs. The use of MSCs obtained from adipose tissue in treating alopecia is a two-component process: freshly derived primary multipotent MSCs that are part of the stromal vascular fraction (SVF), referred to as adipose-derived stromal vascular cells (ADSVCs) or adipose-derived regenerative cells (ADRCs), and the isolated and cultured pure MSCs, termed AD-MSCs [50]. SVF is obtained by removing fat cells from subcutaneous adipose tissue and is composed of peripheral blood-derived cell groups such as macrophages and neutrophils and cell groups such as vascular endothelial cells and ADSVCs or ADRCs. SVF plays an important role in the repair and regeneration of chronically damaged tissue, and it has the potential to promote hair growth by enhancing the capacity of DP to grow or regenerate hair as indicated in the NCT02729415 and NCT02865421 clinical trials. Recent reports indicated that ADRCs were safe and effective for treating AA [50]. In the report, 19 of 20 patients exhibited an increased hair diameter and significantly increased hair density within 3–6 months of treatment. In addition, the impact of ADRCs on hair growth was investigated by combining it with adipose tissue in the transplantation procedure (similar to the cell-assisted lipotransfer reviewed in Ref. [50]) in a study of six patients with MPHL or FPHL [80]. The study results revealed a statistically significant 23% increase in the mean hair count, versus a 7.5% increase in patients treated only with non-assisted adipose tissue.

A review by Owczarczyk-Saczonek et al. [66] re-emphasized several previously reported important details on the use of AD-MSCs in alopecia treatment. First, the interfollicular dermal macroenvironment composed of HFs surrounded by subcutaneous adipocytes and skin is important for maintaining normal cell growth in the bulge and HFs. Second, AD-MSCs secrete growth factors that play critical roles in the activation of epidermal stem cells and hDPCs, summarized in Table 2. Third, through direct interactions between the cells and secretion of PGE2, leukemia inhibitory factor, and kynurenine, AD-MSCs exert immunomodulatory and immunosuppressive effects on epidermal SCs and hDPCs. Fourth, it is suggested that adipose tissue is essential to the extension of anagen because of the ability of progenitor cells, which were adipocytes in the transition from telogen to anagen, to proliferate and (ii) the significant increase in the thickness of the subcutaneous adipocyte layer during the anagen phase versus that in the telogen phase. Finally, AD-MSCs stimulate HF cells via peroxisome proliferator-activated receptors. Conversely, mature adipocytes negatively affect the proliferation of HFs and the surrounding fibroblasts in cocultures. Several studies evaluated the effect of AD-MSCs on alopecia. Zanzottera et al. [61] used the Rigenera® device to prepare autologous AD-MSCs and applied them to the scalps of three patients with AGA. The patients were followed up monthly, and they exhibited more accelerated healing of the AD-MSC transplant-induced wounds and improvements of hair growth with a shorter telogen phase after 2 months of treatment. In Japan, the use of AD-MSCs on the scalps of patients with alopecia is mostly performed by beauty or cosmetic practitioners as a class II risk approved regenerative product in accordance with the PMD Act [57].

3.1.1.2. Treatment of alopecia using BM-MSCs. BM-MSCs have also been explored. In 2018, Elmaadawi et al. [62] reported findings from a study on the safety and efficacy of autologous BM-MSCs in comparison to FHSCs in 20 patients with AA and 20 patients with AGA. In this study, each patient received one intradermal dose of either BMMCs or FHSCs, and the impact was evaluated via immunostaining and digital dermoscopy after 6 months. No study patients experienced any side effects, and all subjects displayed significantly improved hair growth with no significant difference between the groups.

3.1.1.3. Treatment of alopecia using hUCB-MSCs. hUCB-MSCs have proven useful in tissue repair and regeneration. Unlike AD-MSCs and BM-MSCs, it is reportedly easier to obtain sufficient numbers of hUCB-MSCs for transplantation because, in comparison with the former two cell types, more SCs can be acquired from the umbilical cord [81]. Moreover, a study that utilized allogenic, minimally manipulated hUCB-MSCs reported for the first time the successful treatment of AA and alopecia universalis [82]. hUCB-MSCs were isolated from the umbilical cord, immediately frozen, and stored at −197 ºC without any other manipulations including cell culture. Minimal manipulation of SCs reportedly results in safer and better

| Table 2 | Summarizes the activities of different AD-MSCs and the MSC-derived paracrine factors as reviewed in [59,65]. |
|---|---|
| Paracrine factor | Activity on hair growth |
| VEGF | Improves perifollicular angiogenesis, resulting in increased size of HFs and shafts. |
| HGF | Activators enhance the proliferation of follicular epithelial cells |
| EGF | Improves the activity and growth of follicle outer-root sheath cells by activating Wnt/β-catenin flagging |
| PDGF | Induces and maintains anagen phase of hair cycle. |
| IL-6 | Is involved in wound-induced hair neogenesis through STAT3 activation |
| IGF-1 | Improves the migration, survival, and proliferation of HF cells |
| IGFBP1–6 | Manage the effect of IGF-1 and its connection with ECM proteins at the HF level |
| TGF-β | Stimulates the signaling pathways that manage the hair cycle |
| KGF (FGF-10) | Stimulates proliferation and differentiation of early progenitor cells within HFs. Induces anagen phase in resting HFs. |
| FGF-1, FGF-2 | Induces anagen phase in resting HFs. |
| bFGF | Improves the advancement of HFs |
| BMP | Maintains the proper identity of DPCs |
| BMP1a | Maintains the DPC phenotype |
| M-CSF and receptor | Is involved in wound-induced hair growth |
| Wnt3a and receptor | Is involved in HF advancement through β-catenin flagging |
| PGE2 | Stimulates anagen in HFs |
| PGE2 analogs | Enhance the change from telogen to anagen. |
proliferation and differentiation capacities than long-term cultured MSCs [83,84].

3.1.1.4. Treatment for alopecia using hDPCs. hDPCs are differentiated dermal cells at the base of HFs that are surrounded by DSCs. Both hDPCs and DSCs are essential for hair growth and regeneration; however, hDPCs can stimulate HFSCs, and they are believed to play a major role in regulating the cycling of HFs [5]. DPCs with specific marker molecules, such as CD133+ DPCs and versican+ DPCs, can induce HF regeneration. Specifically, CD133+ DPCs can produce Wnt ligands and mediate epithelial–mesenchymal interactions (EMIs) to promote adult HF growth and regeneration [5]. Wnt/β-catenin signaling in DPCs controls other signaling pathways, such as fibroblast growth factor 7 (FGF7) and FGF10 pathways, which regulate the epithelial cell growth HFs [85]. Recently, Ji, et al. [5] reviewed the mechanism of HF regeneration with DPCs and indicated that DPCs were demonstrated to induce HF formation when implanted into recipient non-hairy skin animals. In addition, manipulated and minimally manipulated DPCs could form new HFs when in contact with the epithelium. Therefore, on several occasions, DPCs were cocultured with other cell types, such as the two-dimensional (2D) juxtaposition of other epithelia, cultured epithelial cells; keratinocytes, corneal epithelium, and amnion epithelium, to regenerate HFs. Moreover, the report also indicated that the combination of DPCs and skin epithelial SCs promoted functional HF regeneration in vivo [5]. Nonetheless, experimental studies demonstrated that DPCs dramatically lose the ability to induce new HF formation in conventional 2D cell cultures. However, the use of hanging drop and sphere culture stimulates DPC morphologically and supports their functionality. Culturing DPCs in a 3D microenvironment restores the expression of versican and increases the main development cell signaling of HFs [85].

Multipotent MSCs from the HF bulge region reportedly depend on DSCs to influence the hair cycle [86]. It is also known that transplanting dermis fibroblasts into the dermis reconstructs the dermis, and it is believed that transplanting dermis fibroblasts into the scalp can improve hair regeneration. Tsuboi et al. [87] conducted a randomized study of 50 patients with MPHL and 15 patients with FPHL who received a single injection of autologous DSCs (3.0 × 10^3, 1.5 × 10^5, or 7.5 × 10^5 DPCs/ML) or placebo in four distinctive scalp regions. The study patients were followed up after treatment every 3 months for 1 year. The results revealed significant increases in the total hair density and cumulative hair diameter for patients who received 3.0 × 10^5 DPCs at 6 and 9 months after treatment, suggesting that a minimal number of DPCs is potentially safe and effective in both MPHL and FPHL. In Japan, dermis fibroblast transplantation into the scalps of patients with alopecia is also mostly implemented by beauty or cosmetic practitioners as a class II risk approved regenerative product in accordance with the PMD Act [69].

3.1.1.5. Treatment for alopecia using human iPSCs (hiPSCs). The clinical application of hiPSCs is progressing, with cell therapies under clinical investigation for several ailments [88], especially the use of iPSC-derived products with less risk such as iPSC-MSCs [85]. Currently, it is possible to reprogram somatic cells from a person under clinical investigation for several ailments [88], especially the use of iPSC-derived products with less risk such as iPSC-MSCs [85]. Preclinical studies in rodents utilized the knowledge of stimulating HF morphogenesis and regeneration with organized EMIs and successfully generated HF structures after transplanting various combinations of keratinocytes with DPC hair-generating cells into the animals. The study also revealed that adopting epithelial or dermal HFSPCs resulted in higher regeneration efficiency, suggesting the need to develop means of reproducing real EMIs in HF formation and potentially use HFSPCs and hiPSCs for human HF regeneration. Although in the non-clinical research stage, hair regeneration using hiPSCs may lead to treatment for scarring hair loss, in which the structure of HFs is completely lost [91].

3.1.2. Cell-free therapies
There is increasing interest in the use of the MSC secretome, which comprises bioactive materials, such as growth factors, cytokines, nucleic acids, and other trophic factors playing important roles in regulating the HF cycle and regeneration [92]. In addition, up to 80% of the regenerative properties of transplanted MSCs are reportedly based on paracrine factor signaling [93]. The MSC secretome comprises encapsulated EVs and soluble factors in CM.

3.1.2.1. MSC-derived encapsulated EVs. EVs are categorized according to their diameter as follows: apoptotic bodies, >1000 nm; microvesicles, 100–1000 nm; and exosomes, 30–150 nm. EVs comprise various subcellular particles including growth factor receptors, ligands, adhesion proteins, mRNAs, microRNAs (miRNAs), long non-coding RNAs, second messengers, metabolites, and lipids [94]. However, only miRNAs, mRNAs, and proteins have verified roles in EV-mediated tissue repair and regeneration processes through related specialized signaling pathways, such as mitogen-activated protein kinases or extracellular signal-regulated kinases (MAPK/ERKs), Wnt/β-catenin, phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt), Notch, TGF-β/Smad, signal transducer and activator of transcription, Hedgehog, Ca2+/calmodulin-dependent protein kinase II, and Ephrin A3 signaling [95]. miRNAs are small non-coding RNA molecules of 19–24 nucleotides that target mRNA to regulate post-transcriptional gene expression. EVs containing miRNAs and mRNA, such as miR-124, miR-9/9*, miR-1, miR-133a, and miR-146a, have been identified as regulators of cell proliferation, differentiation, and apoptosis during tissue regeneration [95]. The protein component mainly refers to growth factors, and Gentile and Gavrilovich [52] recently described the various growth factors in EVs with critical roles in hair growth as summarized in Table 2.

3.1.2.2. MSC-derived exosomes. MSC-derived exosomes are secreted through paracrine signaling, and they facilitate cell-to-cell transportation and communication through transcription factors, cytokines, and nucleic acids [93]. Exosomes play important roles in modulating paracrine signaling, and DP cell-derived exosomes may be crucial in HF regeneration [96]. Promising results were reported in preclinical studies, and exosomes might be paramount in treating AA related to autoimmune diseases such as SLE. Cho et al. [97] demonstrated in a mouse model of atopic dermatitis that exosomes from human AD-MSCs reduced the levels of IgE and counts of eosinophils, infiltrated mast cells, and CD86+ and CD206+ cells. In another study using a skin-defect mouse model, UC-MSC-derived exosomes prevented scar tissue formation and reduced myofibroblast accumulation by inhibiting the TGF-β/SMAD2 pathway [98]. It has also been reported that UC-MSC-derived exosomes accelerate re-epithelialization with increased cycleratin-19, proliferating cell nuclear antigen, and collagen I expression in vivo [99]. In a specific study related to autoimmune conditions, MSC-derived exosomes reduced the levels of pro-inflammatory cytokines and promoted
the induction of Tregs in an experimental autoimmune encephalomyelitis model of multiple sclerosis [100]. However, no clinical studies of EVs or exosome-based therapies for hair growth are reported [92].

A recent study identified the involvement of perifollicular macrophages in skin epithelial SC activation as an additional signal that regulates HF growth [101]. Another study found that macrophages induce HF growth through TNF-α/β-catenin signaling in leucine-rich G-protein-coupled receptor 5 (HSC) [101]. Human perifollicular macrophages maintain anagen (making the HF stronger) in humans by activating DPCs through Wnt protein secretion [101]. Macrophage EVs promote proliferation and Wnt/β-catenin signaling pathway activation in DPCs [101]. Macrophage EVs enhance the hair-inductive properties of DPCs by increasing the levels of hair-inductive proteins and survival/proliferation markers [101]. Further experiments revealed that macrophage EVs promote hair growth by stimulating VEGF and keratinocyte growth factor (KGF) in DPCs [101]. Macrophage EVs accelerate hair growth by increasing the number of HFs and dermis thickness. Macrophage EVs could be excellent candidates for stimulating hair growth in humans because their isolation from the same patients is relatively simple and less invasive than that of MSCs from adipose tissue or bone marrow.

3.1.2.3. MSC-derived CM. Emerging evidence illustrates that MSC-derived CM, consisting of cultured MSC-nutrient-rich ECM [102], can effectively induce hair regeneration with additional benefits. CM is not associated with GVHD, a common concern of cell-based tissue regeneration. An in vivo study in mice demonstrated the ability of the MSC secretome to promote epidermal cell proliferation and attenuate macrophage infiltration and ROS production, which activate matrix metalloproteinase (MMP) and degrade collagen tissue [104]. In addition, a recent study on the efficacy and safety of AD-MSC-CM was conducted in 12 patients with FPHL, 13 patients with MPRH, and 1 male patient with both AGA and AA. CM was obtained from ADSCs cultured under hypoxic conditions, and CM obtained from normoxic ADSCs served as the control. All of the different aspects of media were applied every 3–5 weeks using nappage and papule injection methods. The results illustrated that, unlike the control findings, all subjects treated with CM induced under hypoxic conditions displayed statistically significant improvements in hair growth after four treatment sessions over a period of 3–4 months [105]. In another study, 22 patients (11 men and 11 women) with alopecia received hypoxia-induced ADSC-CM injections every 3–5 weeks for six sessions. Ten patients (eight men and two women) treated with a placebo comprised the control group. Statistically significant increases in hair numbers in both genders were demonstrated with negligible post-procedural pain following the physical assessments before and after trichogram in the treatment group versus the control group [106]. Further studies with ADSC-CM evaluated its efficacy in 27 patients with FPHL [107]. Unlike the previous studies, this study used a microneedle roller to apply ADSC-CM weekly for 12 consecutive weeks. Phototrichographic analysis revealed statistically significant increases in both hair density and hair thickness with no serious adverse events in all subjects. In a study by Narita et al. [108], the efficacy of ADSC-CM was evaluated in 40 patients (21 men and 19 women) diagnosed with alopecia. The patients were administered intradermal ADSC-CM monthly for 6 months and followed up every after 2 months. The results revealed significant increases in hair density and the anagen hair rate including the dermal echogenicity and thickness of the treated scalp. In 2006, the use of mesotherapy for alopecia treatment using BM-MSCs and AD-MSCs was announced in Europe. A ripple effect of using cytokines to treat alopecia was later observed in the US and Asia. Mesotherapy is defined as a non-surgical, minimally invasive procedure through intradermal or subcutaneous injection to permit drugs to avoid the stratum corneum barrier and directly affect tissues rapidly, thereby enhancing pharmacological efficacy [109]. Mesotherapy has different aesthetic indications including alopecia, and an injected mixture (cocktail) reportedly comprises various botanic extracts, drugs, or bioactive substances including different cytokines. A recent systematic review by Tang et al. [110] identified 253 men and 274 women who participated in six randomized control trials, two nonrandomized controlled trials, and three observational studies of mesotherapy for treating AGA. The review concluded that mesotherapy was effective against AGA, although inadequate sample sizes and insufficient comparisons with other treatments were noted. Future research was recommended. In 2008, the “Japan Hair Re-Generative Medical Association” was formed. Later that year, the Japan Medical Hair Regeneration Study Group introduced a support treatment for study of laser rejuvenation in both men and women (AAPE®). AAPE® reportedly contains more than 150 types of growth factors, cytokines, vitamins, and blood circulation promoters. It supplements HFs with these regenerative components and activates HFSCs for hair regeneration. Compared to the features of hair and autologous SC transplantation, HARG therapy has the advantages of high safety and lower invasiveness. HARG therapy has not been studied in large-scale clinical trials [112].

hUC-MSC-CM is a collection of many paracrine factors, and its effectiveness against AGA was reported in the NCT03676400 clinical trial. In addition, a recent study described the hair growth-promoting effects of hUCB-MSCs and factors responsible for hair growth [113]. The study reported, “hUCB-MSCs CM increased the viability and upregulated hair induction-related proteins of hDPCs in vitro.” Moreover, IGF-1 and VEGF expression was increased in hUC-MSC-CM, and IGFBP-1 co-localization with IGF-1 promoted cell viability; increased VEGF secretion and alkaline phosphatase, CD133, and β-catenin expression; and promoted hDPC 3D spheroid formation, suggesting that hUCB-MSC-CM promoted hair growth via a paracrine mechanism. Thus, MEDIPost Co., Ltd in Korea has displayed interest in the use of UC-MSC-CM in treating alopecia. Recently, the company developed a product called NGF-574H, which is currently rated excellently by the Ministry of Health and Welfare of Korea; and it displayed equivalent efficacy as minoxidil in treating alopecia in a reportedly completed NCT03676400 clinical trial as described [114].

3.1.2.4. Skin cell secretory protein (hair-stimulating complex [HSC]). HSC is a growth factor-soluble preparation developed by Histogen that is produced by purifying a cell-conditioned medium enriched with growth factors such as KGF, VEGF, and follistatin [115]. Growth factors, especially follistatin, are involved in SC signaling for HF proliferation, and they are important for hair growth and HF stimulation during telogen. In a phase Ib/IIa clinical trial (NCT01501617) comparing HST-001 (HSC preparation) with placebo in male patients with AGA, hair growth was observed in 84.6% of patients. In another study of MPRH, patients treated with HST-001 exhibited statistically significant improvements at week 26 (NCT04435847). Additionally, both trials demonstrated the safety and tolerability of HSC with no reports of serious adverse events.

The aforementioned evidence-based reports indicate the great potential of the SC-derived secretome for treating alopecia.
However, some limitations of the secretome have been observed, and immediate action is needed to improve treatment outcomes in the clinic. First, without persistent efforts to standardize production, the types and levels of factors in the secretome are highly variable, leading to inconsistent treatment outcomes [93]. Second, the regenerative factors in the secretome are associated with high turnover and easy depletion in vivo, necessitating the administration of large quantities more frequently to achieve the desired treatment outcome [116].

### 3.2. PRP therapy for alopecia

PRP is prepared using highly variable protocols. However, in principle, PRP is obtained by centrifuging autologous blood collected from patients under sterile conditions and on anticoagulants as previously described [117]. Briefly, after the initial spin, the intermediate layer of PRP is collected with a sterile syringe, and the first layer, which is the buffy coat or platelet-poor plasma layer, and the red blood cell pellet are discarded. A second spin of the collected plasma layer and addition of platelet α-granule release activators, such as calcium chloride or calcium gluconate, may be considered to concentrate and activate the PRP, respectively, before use. PRP is rich in growth factors known to play critical roles in cell proliferation and differentiation as well as other trophic functions. The main growth factors include PDGF, TGF-β1, TGF-β2, VEGF, bFGF, EGF, IGF-1, IGF-2, and IGF-3, and HGF [118]. PRP is believed to stimulate hair growth by promoting vascularization, angiogenesis, and extension of the anagen phase by growth factor-mediated activation of Wnt/β-catenin, ERK, and Akt signaling pathways, which induce cellular proliferation and differentiation in HFs [27]. PRP therapy is of increasing interest in regenerative medicine, and many clinical investigations have reported promising outcomes regarding hair growth and regeneration [27]. Thus, autologous activated PRP (AA-PRP) and autologous not-activated PRP have been already extensively and successfully applied by dermatologists and plastic surgeon experts to manage various conditions including hair growth [27]. In Japan, PRP therapy for scalp alopecia can be received as a type III regenerative medicine in accordance with the PMD Act [57].

PRP offers an effective treatment for AGA. Unlike FDA/EMA-approved conventional drug treatments for alopecia, autologous PRP therapy is safe and cost-effective, and it requires only short intervals of treatment [119]. A recent study comparing hair count and hair density in patients with scalp alopecia revealed significant improvements in PRP-treated scalp versus placebo-treated scalp, and PRP was well tolerated and more effective against hair loss [120]. PRP treatment was found to be effective against AA [121], and in patchy-type and ophiasis-type steroid-resistant AA, PRP induced hair regrowth and reduced the amount of vellus and dystrophic hairs with complete remission in 60% of patients [122]. The combination of PRP with MSCs provides a promising approach for treating alopecia through the positive impact of PRP on homing. For instance, Butt et al. [123] evaluated the efficacy of injectable autologous PRP and SVF in 11 patients with AGA. The patients were assigned to receive only PRP or a mixture of SVF and PRP (SVF-PRP group). Treatment was administered twice over a 4-week interval, and evaluation was performed 6 months after treatment. The mean hair density increased by 21.5% in the PRP group, versus 51.6% in the SVF-PRP group. A reduction in the pull test was observed in both groups, with a more significant reduction in the SVF-PRP group. There were also significant improvements in the physician and patient assessment scores in the SVF-PRP group. An additional study by Stevens et al. [124] evaluated a similar PRP combination in 10 male patients with AGA, albeit with a single dose, and reported significant increases in hair density at 6 and 12 weeks after treatment. In addition, in a recent review by Stevens et al. [124], the authors recommended the synergistic use of PRP with conventional therapies, such as minoxidil, spironolactone, and finasteride. Reportedly, PRP has no impact on the hormone-related pathogenesis of AGA [124]. Based on published studies, Stevens et al. [124] also suggested that for optimal results, the production of pure AA-PRP at 3–6-fold its mean concentration in whole blood for platelet enrichment and granulocyte minimization and the use of a more efficient subdermal depot bolus injection technique are necessary. The studies in the review indicated several findings. First, in subdermal AA-PRP administration, active growth factor secretion starts within 10 min of activation with the release of >95% within 1 h, and the synthesis continues for 7 days. Second, the lack of standardized PRP production results in samples with varying amounts of platelets, leukocytes, erythrocytes, and growth factors, which could induce different biological responses. Several studies demonstrated that a platelet count up to 6-fold higher than the basal count promotes tissue regeneration [125]. Third, the impact of granulocytes and red blood cells in PRP on AGA treatment remains a subject of contention. The presence of leukocytes might improve the effectiveness of PRP through protection against infection and increased growth factor release, which leads to angiogenesis, matrix production, and cell proliferation [126]. By contrast, leukocytes can cause matrix degradation through increased MMP levels, and the existence of neutrophils and red blood cells could result in increased levels of ROS and pro-inflammatory cytokines such as TNF-α, leading to increased inflammation and tissue destruction [126].

Despite encouraging reports of promising alopecia treatment outcomes with PRP, larger, more controlled clinical trials with longer follow-up periods and standardized protocols and dose regimes are required [117].

### 4. Conclusion and further perspectives

Several strategies for restoring abnormal or lost hair in regenerative medicine are currently being explored, and cell-free therapies including the MSC-derived secretome (EVs and exosomes) and PRP remain attractive emerging options. Increasing evidence suggests that EVs and PRP are highly feasible intervention approaches for several diseases because of their multiple physiological functions and clinical application advantages. The common therapeutic potential of both strategies is based on their rich composition of various paracrine factors with multifarious functions. Compared to MSC cell-based therapy with superior homing and pluripotent differentiation potential, the paracrine functions of MSC-derived EVs and PRP have been demonstrated to play a more critical role in restoring injured tissues or organs such as HFs. Multipotential MSCs are abundantly available in most perinatal and postnatal body tissues. However, cell-based therapies are limited by the increased risks of adverse events related to their administration, such as embolism, respiratory compromise, immune response disorders like GVHD, and longer-term complications including the risk of ectopic tissue formation and malignancy. Moreover, the therapeutic effect is minimized over time by cell manipulations such as prolonged passes in culture, which causes senescence for some tissue-specific derived MSCs. Conversely, several factors generally render both MSC-derived EVs and PRP safer including, inter alia, the inability to self-replicate, which significantly reduces the potential for endogenous tumor formation, and low immunogenicity together with low embolus formation, which minimizes the development of GVHD and fetal respiratory compromise. In addition, both therapies retain their potency upon technological manipulation and longer storage. MSC-derived EV and PRP manipulations are easier and less expensive.
and long-term storage provides an opportunity to develop ready-to-use products suitable for emergency healthcare and applicable in resource-limited settings such as developing countries [127,128].

Nevertheless, the clinical application of MSC-derived EV and PRP therapies for various diseases including alopecia is not completely feasible because of some limitations, including the heterogeneous MSC-derived EV and/or impure PRP composition; limited technology for large-scale production; a lack of optimal approaches for long-term storage; non-efficient determination of product concentration and quality control, purification, and transplantation conditions; and unresolved long-term safety issues. We believe that within the next decade, substantial progress will be achieved through global efforts to overcome these limitations. Such efforts include ongoing and future statistically robust randomized controlled clinical trials, innovative advances in manufacturing technology, proactive academia, and industry collaborations, and compliance with the regulatory and safety considerations as discussed in the International Society of Extracellular Vesicles position paper on the application of EV-based therapeutics in clinical trials [129]. For instance, the starting cellular material produced or iso-
paper on the application of EV-based therapeutics in clinical trials discussed in the International Society of Extracellular Vesicles position technology, proactive academia, and industry collaborations, and compliance with the regulatory and safety considerations as discussed in the International Society of Extracellular Vesicles position paper on the application of EV-based therapeutics in clinical trials [129]. For instance, the starting cellular material produced or iso-

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