REVIEW ARTICLE

Stem cell in alopecia areata

Bayoumi Eassa, MD, Essam Bakr Abd-El-Aal, MD

Department of Dermatology, Al-Azhar University, Cairo, Egypt

ABSTRACT

Alopecia areata (AA) is a non-scarring, autoimmune, inflammatory, hair loss on the scalp and/or body. Higher rates than expected of antibodies characteristic of other autoimmune diseases have been reported in patients with AA. Although attempts to identify disease-specific autoantibodies for AA in humans have failed. The increased frequency of auto antibodies in AA are reported associations with other autoimmune diseases such as thyroid disorders, vitiligo, pernicious anemia, diabetes, lupus erythematosus, myasthenia gravis, lichen planus, autoimmune polyendocrine syndrome type and celiac disease.

Hair growth depends on the activity of the highly proliferative hair matrix cells in the hair bulb of the inferior segment. The hair matrix cells are supplied by slow-cycling and multipotent stem cells in the anagen phase, the hair follicle keratinocyte stem cells migrate from the bulge and proliferate to participate in hair bulb formation during hair growth. Thus, the adult follicle possesses an innate ability to regenerate its hair-producing apparatus using cells located in specific follicular niches. No cure or preventive treatment for AA has been established, thus treatments are directed toward halting disease activity. Addressing the impressive inflammatory process occurring in AA, corticosteroids have been the most popular treatment modality. Numerous types of stem cells, including embryonic, neural, and mesenchymal bone marrow, all have the capacity to form skin and hair when introduced into blastocysts.This raises the intriguing possibility that these other types of stem cell could be coaxed into forming hair follicles by the proper inductive cells. This has lead the researchers to try stem cell based therapies for the treatment of alopecia areata. The details of which are discussed in this article.

INTRODUCTION

Hair has a great social significance for human beings. Human hair growth activity reflects both physiological and pathological conditions. Normal hair is seen in healthy, young individuals, while baldness and white hair increase in an age-dependent manner.[1]

Hair growth depends on the activity of the highly proliferative hair matrix cells in the hair bulb of the inferior segment. The hair matrix cells are supplied by slow-cycling and multipotent stem cells in the anagen phase, the hair follicle keratinocyte stem cells migrate from the bulge and proliferate to participate in hair bulb formation during hair growth.[2] By contrast, in the catagen and telogen phases, the inferior portion of hair follicles disappears.[3]

Alopecia areata (AA) is a non-scarring, autoimmune, inflammatory, hair loss on the scalp and/or body.[5] Recognized subgroups of this disease include those patients with the complete absence of terminal scalp hair (alopecia totalis or AT) and those patients with total loss of terminal scalp and body hair (alopecia universalis or AU).[6,7] Another pattern of hair loss can be clinically differentiated is the ophiasis pattern, which refers to AA extending along the posterior occipital and temporal scalp margins.[8]

Correspondence: *Dr. Bayoumi Eassa, Department of Dermatology, Al-Azhar University, Cairo, Egypt*

ETIOLOGY AND PATHOGENESIS

The etiology of AA has experienced a continuing evolution since its first mention in 1760. In the late 19th and early 20th centuries, epidemics of AA were reported in orphanages and schools, alleging a parasitic or infectious etiology.[10]

A viral etiology was proposed in the late 1970s but subsequent articles have demonstrated no connection.[11,12] The concept of AA being the result of a nervous disorder was supported by reported associations of AA with emotional or physical stress and trauma.[13] It has been reported that there is a high prevalence of mood, adjustment, and anxiety disorders in patients with AA.[14] A genetic study by Yang et al found that 8.4% of the patients had a positive family history of AA, suggesting a polygenic additive mode of inheritance.[15]

Lenane et al reported four cases of congenital AA, which they followed for 5 years, suggesting that this disease can be classified as acquired or congenital.[16] AA is an organ-specific autoimmune disease with genetic predisposition and an environmental trigger.[17]

Patients with AA have an increased frequency of autoantibodies to follicular structures; however, there is little consistency in which follicular structures are labeled by the antibodies.[18] Higher rates than expected of antibodies characteristic of other autoimmune diseases have been reported in patients with AA. Although attempts to identify disease-specific autoantibodies for AA in humans have failed.[19] The increased frequency of auto antibodies in AA are reported associations with other autoimmune diseases such as thyroid disorders,[20] vitiligo,[21] pernicious anemia, diabetes,[22] lupus erythematosus,[23] myasthenia gravis,[24] lichen planus,[25] autoimmune polyendocrine syndrome type[26] and celiac disease.[27]

Thyroid disorders and vitiligo have the strongest relationship to AA. An incidence rate of 2.3% for thyroid disease has been reported in patients with AA,[11] while vitiligo has been reported to occur in 4.1%.[28]

HISTOPATHOLOGY

For over a century, follicular associated inflammatory infiltrates have been observed in AA.[29] The early stage of AA is characterized by an increased number of the catagen and telogen follicles, the presence of inflammatory lymphocytic infiltrate in the peribulbar region ("swarm of bees") and eosinophils in the stellae.[30]

The hair matrix is infiltrated by lymphocytes and there is also pigment incontinence, matrix cell necrosis, and vacuolar damage. The inflammatory infiltrate is especially prominent in terminal hair follicles, the bulbs of which are located in the subcutaneous tissue. The infiltrate is composed of CD4+ and CD8+ T lymphocytes.[31]

Degeneration of the lower follicular keratinocytes, melanocytes, Langerhans cells, and dermal papillae can be seen.[32] Immunofluorescence studies have shown deposits of C3, IgG, and IgM along the basement membrane of the inferior part of the hair follicle.[33]

CLINICAL FINDINGS

Alopecia areata most commonly manifests as a sudden loss of hair in well-demarcated, localized areas. The lesion is usually a round or oval patch of alopecia and may be isolated or numerous. The patch of alopecia usually has a distinctive border where normal hair demarcates the periphery of the lesion. The scalp is the most common site affected by AA (90%).[9,20]

The affected skin appears normal with no epidermal alteration grossly visible such as scaling or follicular abnormalities.[5] The affected hairs undergo an abrupt conversion from anagen to telogen, clinically seen as localized shedding. Characteristic hairs, known as "exclamation point hairs," may be seen within or around the areas of alopecia. The hairs are tapered toward the scalp end with thickening at the distal end. Hair pull tests conducted at the periphery of the lesion may be positively correlated (six hairs or more) with disease activity, thus, hair loss progresses in a circumferential pattern. Often, distinct patches merge to form larger patches.[34] Upon re-growth, hairs will often initially lack pigment resulting in blonde or white hairs.[35]

Involvement of the nails in the form of nail pitting can also be seen.[5] Other nail features found in AA are trachyonychia, Beau's line, onychorrhexis, thinning or thickening, onychomadesis, koilonychias, punctuate or transverse leukonychia, and red spotted lunula.[34]

TREATMENT OF ALOPECIA AREATA

No cure or preventive treatment for AA has been established, thus treatments are directed toward halting disease activity. Addressing the impressive inflammatory process occurring in AA, corticosteroids have been the most popular treatment modality. Glucocorticoid treatment has an overarching anti-inflammatory effect.[36]

Several methods of corticosteroid administration have been used, notably intralesional injection, topical, and systemic therapy. Further attempts have been made at not only reducing inflammation, but to also stimulate hair growth, such as minoxidil. Popular methods outside the United States have been to stimulate alternative inflammatory pathways in an attempt to stop the cycle of autoimmune inflammation. Currently, newer treatments targeting the immune system are being explored for use in AA.[37]

A. TOPICAL TREATMENT

1. Intralesional corticosteroids

Although intralesional corticosteroids (ILCSs) have been used in the treatment of AA for about 50 years, there are no published randomized controlled trials.[38] For circumscribed AA involving less than 50% of the scalp, intralesional corticosteroids are the first-line approach.[39]

Triamcinolone acetonide is administered using a 0.5-inch long 30-gauge needle in multiple 0.1 ml injections approximately 1 cm apart.[39,40] The preferred method of injection is to avoid superficial administration and to penetrate the deeper dermis.[41]

Concentrations have ranged between 2.5 and 10 mg/ml, but it appears that 10 mg/ml is the preferred concentration for the scalp.[19,42] Lower concentrations of 2.5 mg/ml are used for the eyebrows and face. A maximum of 3 ml total on the scalp in one visit is recommended. Initial results of intralesional treatment are often seen in 1-2 months. Additional treatments are repeated every 4-6 weeks. It is important to avoid transient atrophy of the injected area that is often seen in the setting of too much injected amount, too frequent injections, or insufficient depth of injection. Pain limits the practicality of this method of treatment in children less than 10 years of age.[40]

One possible reason for the lack of response or resistance in some AA patients after 6 months of therapy is low levels or decreased expression of the enzyme thioredoxin reductase -1 (TR) in the outer root sheath, an enzyme that "activates" the

glucocorticoid receptor (GCR).[19]

2. Topical corticosteroids

Several forms of topical corticosteroids have been reported to exhibit varying levels of efficacy in AA. Some of the topical therapies have included fluocinolone acetonide cream,[43] fluocinolone scalp gel betamethasone valerate lotion, 10 dexamethasone in a penetration-enhancing vehicle,[44] desoximetasone cream,[45] halcinonide cream,[46] and clobetasol propionate ointment. Tosti et al demonstrated a response rate in 28.5% of patients with either AT or AT/AU refractory to other topical treatments using clobetasol propionate 0.05% ointment under occlusion. Unfortunately, 37.5% of the responders experienced relapses of AA and were not able to maintain hair re-growth despite treatment.[47] Because of its ease of application in more widespread lesions, topical corticosteroids remain a very convenient method of treating patients initially presenting with more widespread hair loss. They remain a very good option in children because of their painless application and wide safety margin.[10,47]

3. Minoxidil

Minoxidil was first introduced as an antihypertensive agent, and its side effect of hypertrichosis lead to its use as treatment for various forms of alopecia. Although minoxidil has been used as a hair re-growing treatment for more than 20 years, its mode of action is not fully understood. Many mechanisms of action have been proposed, including vasodilatation,[48] angiogenesis,[49] enhanced cell proliferation,[50] and potassium channel opening. [51] There are some reports indicating that minoxidil also has some immunosuppressive effects.[52]

Patients consistently resistant to minoxidil treatment often suffer from severe AA, AT, or AU.[53] Younger patients appear to have a better response to minoxidil than older patients. Several of these studies attempted to further refine the concentration of minoxidil, resulting in more successful results.[54]

Minoxidil has also been studied in combination with anthralin,[55] topical betamethasone propionate, and prednisone.[56] Fiedler et al studied 51 patients with severe, recalcitrant AA treated previously with anthralin and/or minoxidil. They found that 11% of the patients had a cosmetically acceptable response.[55] Also, major improvement was observed in patients treated with combination of minoxidil and 0.05% betamethasone proprionate.[56]

Contact dermatitis can occur in 6% of patients using 5% minoxidil solution.[57] Because minoxidil 5% foam does not contain propylene glycol (a potential irritant), the incidence of pruritus with it is much reduced compared to the 5% minoxidil solution (1.1% vs 6%).[58] Hypertrichosis (facial hair growth) has been a reported side effect in 3% of patients.[59]

4. Anthralin

Anthralin is presumed to elicit hair growth through its irritant contact properties. Its exact mechanism of action is unknown, but is believed to be through immunosuppressive and anti-inflammatory properties via the generation of free radicals.[60] Cosmetically acceptable hair re-growth has been reported in 20-25% of 68 patients in a controlled study[61] and as high as 60% of 32 patients in an uncontrolled study.[62] In a half-sided, controlled study, 0.1% anthralin was not found to induce any differences between the treated and untreated sides.[63]

5. Topical immunotherapy (contact sensitizers)

Topical immunotherapy is defined as the induction and periodic elicitation of an allergic contact dermatitis (ACD) by applying potent contact allergens to the affected skin. It appears that contact sensitizers act through immunomodulation of the skin and its appendages at several different points.[64]

Many theories have been suggested for the mechanism of action of topical sensitizers. These include antigenic competition,[65] perifollicular lymphocytes apoptosis,[66] and changes in the peribulbar CD4/CD8 lymphocyte ratio (4:1 in untreated progressive AA to 1:1 in DPCP-responding patients).[67] Hoffman et a hypothesized that interleukin-10 (IL-10) secretion from basal keratinocytes or lesional T- cells after DPCP application results in an inhibitory effect on lesional T- lymphocytes.[68]

Dinitrochlorobenzene (DNCB), was the first sensitizer used for the treatment of AA.[69] Early in its therapeutic history, DNCB was found to have mutagenic effects on the Ames test, and has therefore been abandoned as a viable treatment choice, although there is some controversy surrounding the clinical applicability of the Ames test.[70]

Another topical sensitizer is squaric acid dibutyl ester (SADBE), which it has been reported to be useful in treatment of AA, the results showed a cosmetically acceptable hair re-growth in 28% to 80% of patients.[71] Some drawbacks to SADBE's practical use are its requirement for refrigeration and its relative instability in acetone compared to the more commonly used contact sensitizer diphenylcyclopropenone.[72]

Diphenylcyclopropenone (DPCP) was first synthesized in 1959,[73] and is used today in Europe and Canada. Ultraviolet (UV) light and heat cause degradation of DPCP. It is therefore used with the standard solvent acetone, a strong UV light absorber. This agent is a potent contact sensitizer, inducing an allergic response on the scalp in 98-99% of AA patients.[74] There have been several studies conducted testing the efficacy of DPCP in AA, with re-growth rates of 4% to 85%.[75]

Adverse effects of topical immunotherapy include pruritus, mild erythema, scaling, and postauricular lymphadenopathy.[40,76] Reported undesired side effects include contact urticaria,[77] postinflammatory hyper- and hypo-pigmentation, erythema mutliforme,[78] facial or eyelid edema,[79] fever, flulike symptoms, anaphylaxis,[80] "dyschromia in confetti,"[79] and Vitiligo.[81]

6. Calcineurin inhibitors (Tacrolimus)

Tacrolimus is a topical calcineurin inhibitor that inhibits transcription following T-cell activation of several cytokines including interleukin-2, interferon-γ and tumor necrosis factor-α.[82] Yamamoto et al reported in their findings that tacrolimus stimulated hair growth in mice,[83] although subsequent studies showed conflicting results.[84]

7. Capsaicin

The idea of using capsaicin in AA emerged from the theory of nervous system and neuropeptide role in the development of the disease. Capsaicin can release substance P (SP) and calcitonin gene-related peptide (CGRP), and after repeated application, it depletes neurons of SP.[85] Capsaicin cream 0.075% resulted in vellus hair re-growth in two patients after 3 weeks of treatment. Both patients had burning pain sensation with treatment.[86]

8. Prostaglandin analogues

Latanoprost, a prostaglandin F2a analogue, and bimatoprost, a synthetic prostamide F2a analogue, are used to reduce intraocular pressure in open angle glaucoma patients. Eyelash hypertrichosis is a common adverse effect of their use that has been confirmed in murine hair follicle studies.[87] Prostaglandin F2a and its analogue showed stimulatory effects on murine hair follicles and follicular melanocytes in telogen and anagen phases and enhanced the conversion from the telogen to the anagen stage. Bimatoprost ophthalmic solution (Lattisse; Allergan, Inc, Irvine, CA) received approval from the US Food and Drug Administration for the treatment of hypotrichosis of the eyelashes.[88]

9. Bexarotene

Dermal irritation is a common side effect of Bexarotene 1% gel treatment and was evaluated in a single blinded study involving 42 patients with AA. Five patients (12%) had 50% or more partial re-growth on the treated side, and six patients (14%) had a response on both sides. Some degree of dermal irritation was experienced by 73% of the patients. The mechanism of action is thought to be through immunomodulation and induction of T-cell apoptosis. The efficacy of bexarotene needs to be confirmed in randomized, placebo-controlled trials.[89]

B. SYSTEMIC TREATMENT OF AA

1. Systemic corticosteroids

Reports of systemic corticosteroid use for AA go as far back as 1952.[90] The suggested dosages are 1 mg/kg/day for adults and 0.1-1 mg/kg/day for children.[10,8] The dosages necessary to maintain hair re-growth in AA are between 30 and 150 mg daily.[91] Treatment course can range from 1 to 6 months, but prolonged courses should be avoided secondary to the bone related side effects of these drugs especially when children are treated. Other side effects include a rebound flare-up of the treated disease, acute adrenal insufficiency, fever, myalgias, arthralgias, and malaise; fluid and electrolyte abnormalities, hypertension, hyperglycemia, increased susceptibility to infection, osteoporosis, behavioral disturbances, cataracts, and Cushing syndrome. Its side effect profile in conjunction with the long-term treatment requirements and high relapse rates make systemic corticosteroids a more limited option.[8]

Olsen et al reported that 12% - 28% of patients with 1% to 99% scalp AA, re-grew 50% or more of their hair after a 6-week prednisone taper (starting at 40 mg).[92] Successful therapy with pulsed methylprednisolone (250 mg IV twice daily for 3 consecutive days) resulted in 50% or more re-growth in 65% of the patients after 1 month of treatment for patchy AA was reported by Friedli et al[93] and Assouly et al[94] the authors attributed their success to treating patients with "active" lesions. A lack of efficacy of this drug was shown in patients suffering from refractory chronic AA.[91] Contraindications and side effects should be discussed at length with patients slated for this therapy. Other authors have demonstrated that intravenous dexamethasone pulse therapy 5 mg twice weekly for a minimum period of 12 weeks is safe and effective for extensive AA.[95]

Other ways of administering systemic corticosteroids include alternating daily doses of prednisone,[96] oral prednisolones 300 mg once monthly,[97] intravenous prednisolone 2g as single dose,[94] and dexamethasone 5 mg twice weekly for a minimum period of 12 weeks.[98]

2. Cyclosporine A (CsA)

Cyclosporine A is a common antirejection therapy used in post-transplantation patients which exerts its effect via inhibition of T-cell activation.[99] A common cutaneous side effect is hypertrichosis, which occurs in approximately 80% of patients, possibly as a result of prolongation of the anagen phase of the hair cycle.[100] It also decreases the perifollicular lymphocytic infiltrates, particularly the mean number of helper T-cells.[101] Successful use of systemic CsA in patients with AA has been conflicting.[102]

CsA is a nephrotoxic, hepatotoxic drug, it also causes gingival hyperplasia, headaches, tremors, and hyperlipidemia. Drug interactions and nominal efficacy of CsA make it a poor choice for the use in AA.[99]

3. Methotrexate

Methotrexate in conjunction with low-dose prednisone showed success in 64% of patients with AT/AU in one study. In a retrospective uncontrolled trial of weekly 20-25 mg methotrexate combined with 20 mg/d oral prednisone in 22 AT/AU patients, total recovery occurred in 14 patients (64%).[102] These results need to be confirmed in randomized controlled studies.

4. Sulfasalazine

Sulfasalazine has both immunomodulatory and immunosuppressive actions, including inhibition of T-cell proliferation, natural killer cell activity, and antibody production. Sulfasalazine also inhibits the T-cell cytokines IL-2 and IFN-g and the monocyte/macrophage cytokines IL-1, TNF-α, and IL-6. A few case series have shown some improvement of AA after using sulfasalazine.[103] Ellis et al reported cosmetically acceptable hair re-growth in 23% of patients in a retrospective study.[104]

5. Biologics

These drugs are made up of recombinant cytokines, humanized monoclonal antibodies, and molecular receptors that bind target molecules. These medications reduce the pathogenic T-cells, inhibit T-cell activation and inhibit inflammatory cytokines, suggesting a potential role in the treatment of AA.[105]

Etanercept (Enbrel) is a fusion protein receptor consisting of two human TNF receptors and Fc domain of human immunoglobulin G1. Strober et a administered 50 mg of etanercept twice weekly to patients with moderate to severe AA. They observed no significant hair re-growth after 24 weeks of treatment.[106] Two case reports found patients developed AA while on treatment with etanercept and infliximab (Remicade) (a chimeric (mouse-human) IgG1 monoclonal antibody that binds to TNF-α).[107,108] These findings suggest that TNF-α may not be essential in the mechanism of AA.

A placebo-controlled trial of subcutaneous efalizumab (Raptiva), an anti-CD11a antibody, in 62 patients for 3 to 6 months did not show statistical differences between the efalizumab and placebo groups.[109] There are a few reported cases that have shown either development of AA or complete failure to respond to different TNF-a antibodies, including adalimumab (Humira),[110] infliximab,[111] and etanercept.[112] Higher doses, longer therapy periods, or longer follow-up may be needed.

6. Psychosocial support

AA is associated with high psychiatric co-morbidities (mainly adjustment disorder, generalized anxiety disorder, and depressive disorders).[23] The efficacy of antidepressants in AA treatment has not been evaluated by large-scale random-

ized control trials. In a small trial of eight AA patients treated with 20 mg paroxetine, a selective serotonin reuptake inhibitor (SSRI), and five patients with placebo for 3 months, complete hair re-growth was observed in two patients in the paroxetine group versus one patient in the placebo arm. Four patients in the paroxetine group showed partial hair re-growth.[113]

Willemsen et al showed 75% to 100% hair re-growth in 12 of 21 patients with extensive AA after three to eight sessions of psychotherapy. In the follow-up period (ranging from 4 months to 4 years), the relapse rate was 42%. The small sample size and less than optimum hair re-growth assessment makes the evaluation of some trials of antidepressants difficult. Support groups that involve regular meetings of AA patients and family members can be an invaluable resource for them. Patients can derive emotional support and information that can help them develop positive coping strategies, overall improved quality of life, and increased treatment compliance. The National Alopecia Areata Foundation (NAAF) provides patients and physicians with brochures, research updates, bimonthly newsletters, a pen pal program, sources for scalp prostheses, and many patient conferences. Also, the NAAF supports research and research workshops that add to the scientific knowledge about AA.[114]

7. PUVA

The use of PUVA (psoralen plus ultraviolet light A) is based on the concept that the mononuclear cells and Langerhans cells that surround the affected hair follicles may play a direct pathogenic role and that PUVA therapy can eradicate this inflammatory cell infiltrate.[23,114]

Mohamed et al used topical 8-MOP plus UVA at higher doses (8-42 J/cm^2) in 124 patients with AA and 25 patients with AA totalis or universalis and they found that 85% of patients from the AA group had good or excellent response to the treatment, and 14 patients from AA U group had ≥50% hair re-growth. Side effects included slight erythema and painful burning in patients who did not protect their scalp from sunlight after PUVA exposure. Recurrence of hair loss was noted in eight cases after a period of 10 months to 2 years of treatment.[115]

8. Other phototherapies

A few case series have shown successful results with 308-nm excimer laser in treating patchy AA. The initial fluences were 50 mJ/cm^2 less than the minimal erythema dose. Fluences were then increased by 50 mJ/cm^2 every two sessions. Each patch was treated twice a week for a maximum of 24 sessions. Hair re-growth has been shown in 41.5% of patches.[116] No re-growth of hair was observed in the control patches. Poor results were achieved with AU or AT patients.[117] Further randomized controlled studies are required to evaluate the effectiveness of 308-nm excimer laser.

Stem cells treatment for alopecia areata

In fetal skin, hair follicles develop from two major cell types epithelium and mesenchyme and crosstalk between these two cell populations is critical. As no new hair follicles form after birth, bioengineering new hair follicles may seem unduly challenging. However, unlike other organs, each hair follicle normally regenerates itself cyclically in a manner that recapitulates embryonic hair follicle development.[119]

In the adult, the lower hair follicle reforms itself with each new hair cycle by the interaction of the epithelial stem cells in the bulge with ad-

jacent mesenchymally derived dermal papilla cells. Thus, the adult follicle possesses an innate ability to regenerate its hair-producing apparatus using cells located in specific follicular niches.[119] The successful generation of viable offspring derived from fetal and adult mammalian cells by cloning technology reached a high point in 1997 with the now famous sheep named Dolly born in the United Kingdom.[120] Shortly thereafter, in 1998, human embryonic stem cells were isolated from blastocysts at the University of Wisconsin, Madison. Together with the description of an up to then unknown plasticity of stem cells, the generation of a human embryonic stem cell line fueled a great interest in stem cell biology driven by a promise of repairing and replacing diseased tissues and organs.[121]

An ardent example is spinal cord injury for which stem cell transplantation is discussed as a therapeutic option.[122,123] With the later restriction on federally funded embryonic stem cell research in the United States between 2000 and 2008 and its only recent reversal on March 8, 2009, the research in this country was more focused on adult stem cells.

The skin was and is a natural target of stem cell research because of its large size and easy accessibility. Although the focus was initially on hair follicle stem cells driven by their early discovery in the follicular bulge,[124] stem cells of other lineages were recently characterized in the skin. These include the melanocytic stem cells, which were shown to reside in close proximity to the keratinocytic stem cells of the bulge and whose loss is responsible for hair graying.[125,126] However, the characterization of stem cells in the cutaneous mesenchymal compartments has largely escaped the attention of the majority of the dermatologic community and has only recently taken off. This is surprising because the dermis may represent a larger reservoir for adult stem cells than the epidermis and the hair follicle together.[127] Moreover, the subcutaneous tissue is a source of stromal stem cells with the capacity of multipotential differentiation.[128,129] Both dermis and adipose tissue are viewed as promising alternatives to bone marrow derived stem cell therapies. This makes dermatology a key discipline of mesenchymal stem cell (MSC) research.[130]

Hair follicle stem cells are multipotent, capable of proliferation and able to give rise to all cell types of the hair, the epidermis and the sebaceous gland.[131] Hair follicle stem cells, like other adult stem cells, are thought to be slow-cycling cells or rarely cycling cells, with a superior clonogenicity and proliferative capacity. Unlike these other tissues, however, hair follicle growth (anagen) is interrupted by periods of regression (catagen) and rest (telogen), with subsequent regeneration.[132,133] The entire lower epithelial structure is formed during anagen, and regresses during catagen. The hair follicle stem cells proliferate at the onset of anagen phase and regenerate the new lower follicle. The bulge cells then become quiescent and remain so for the remainder of the hair cycle. As the lower part of the hair follicle cyclically regenerates, hair follicle stem cells are thought to govern this regeneration.[134]

Epithelial stem cells have a high proliferative potential and can proliferate at times of tissue expansion such as wound healing.[124] They have also been implicated in tumorigenesis and their manipulation may have a wide range of applications such as in the delivery of gene therapy, tissue engineering, and treatment of skin diseases and hair loss.[135]

Localization of the follicular stem cells

Most of the hair follicle stem cells responsible for this cyclical regeneration of the lower follicle reside in an area called the hair follicle bulge,[124,134,136] a portion in the outer root sheath, located in the mid portion of the hair follicle, which is at the insertion site of the arrector pili muscle Figure (1).[137] This unique interaction between the bulge and the arrector pili muscle could play an important role in maintaining the phenotype of the bulge cells. Lineage analysis definitively demonstrated that bulge cells give rise to all of the epithelial cell types in the normal regenerating lower follicle during anagen

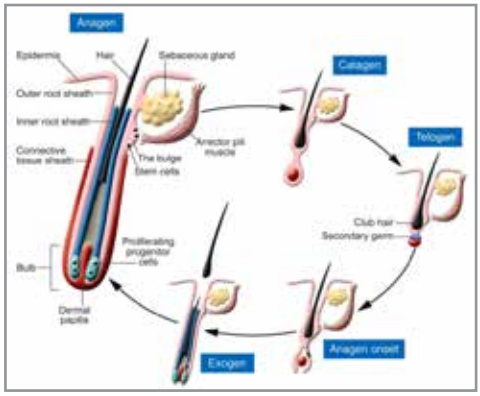

Fig. 1 Hair follicle, Cotsarelis G.[134]

(i.e. the growth phase), in addition to the sebaceous gland and overlying epidermis.[134,136]

In the microscopic anatomy of the follicle, the bulge region can be recognized as a prominent protuberance below the sebaceous gland in vertical sections of murine and human fetal specimens stained with haematoxylin and eosin. In contrast to the bulge of murine follicles, the human adult anagen bulge does not possess distinctive morphological features and is barely prominent. While usually very difficult to see in human skin, characteristic protrusions (follicular trochanter) provide a useful histological demarcation of the human bulge.[138]

The localization of these stem cells to the bulge area may explain why some types of inflammatory alopecia cause permanent loss of hair (cicatricial alopecia) [such as lichen planopilaris (LPP) and discoid lupus erythematosus (DLE)], while others [such as alopecia areata (AA)] are reversible (noncicatricial alopecia). The characterization and manipulation of stem cells have led to a wide range of clinical applications.[139]

Although several lines of evidence have suggested that the bulge region also provides a niche for interfollicular epithelial stem cells, anatomical boundaries, biochemical distinctiveness and global gene expression pattern are ill defined. The lack of distinctive bulge morphology in human hair follicles has hampered studies of bulge cells and epithelial stem cells, and because of that there is a strong need for specific hair follicle stem cell markers to allow easy identification of these cells.[140]

Hair follicle stem cell markers

The function of the bulge region, located at the lower 'permanent' end of the hair follicle, remained unknown until the recognition of a monoclonal antibody to CK15 that specifically targeted keratinocytes in this region.[141] Using this antibody it was apparent that hair follicle bulge cells possess characteristics of stem cells such as multipotency, high proliferative potential and quiescence.[134] Also, it has been shown that CK15 cells are lost in some scarring alopecia's, implying involvement of the bulge region in scarring alopecia.[142]

Hoang M.P. et al found that in cases of scarring alopecia no CK15 expression. Of interest, they found that all of these cases were characterized by marked fibrosis but minimal inflammation.[143]

This is somewhat different from findings in the study by Pozdnyakova and Mahalingam, in which scarring alopecia cases with loss of CK15 typically had moderate to heavy perifollicular inflammation and minimal fibrosis while cases with marked fibrosis showed retention of CK15 staining.[144] Given that CK15 stains the peripheral layer of the ORS above the attachment of the pilar muscle, a possible explanation for the discrepancy is that the inflammation was observed at or above the level of the bulge region Fig.(2).[143]

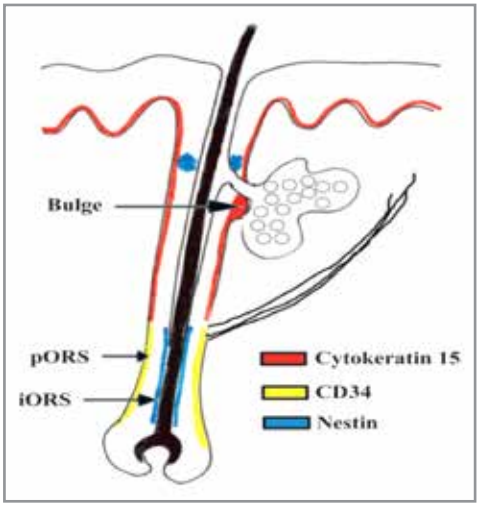

Fig. 2 Stem cells markers, Hoang et al.[143]
pORS: Peripheral layer of outer root sheath.
iORS : Inner aspect of outer root sheath.

To date, the best marker for bulge epithelial stem cells in human hair is cytokeratin (CK15).[145] Human stem cells have been reported to express CK15 selectively throughout all stages of the hair cycle in different types of follicles,[146] with important findings and good correlation between the presence of stem cells and the expression of the surrounding CD8+ T lymphocytes, and CK15 (clone CD8 /144B) preferentially immunostains hair follicle stem cells without staining the other basal cells of the hair follicle.[140]

CD34 expression in the skin is noted in a variety of mesenchymal-derived cells including endothelial cells and dermal dendritic cells, the hair follicle appears to be the only human structure in which expression of CD34 is noted in epithelial cells.[147,148] In the follicle, CD34 expression has been noted in perifollicular cells. Also, CD34 was expressed in the outermost /peripheral layer of the ORS below the attachment of the pilar muscle of human anagen hair follicles but negative expression in the bulge area.[146]

The pattern of staining by CD34 antibody has been reported to be different according to the stage of hair follicle development, with only anagen human hair follicles showing CD34 immunoreactivity, while the catagen and telogen follicles did not show CD34 immunoreactivity in the outer root sheath.[146] CD34 immunoreactivity is found in the outer root sheath below the bulge zone (follicular trochanter) which does not include bulge cells.[140]

In cases of AA, focal nestin expression was noted in the infundibular region above insertion of the sebaceous glands in seven of 32 cases (22%). Nestin was also noted in the innermost aspect of the ORS below the attachment of the pilar muscle in 23 of 23 cases (100%) in which the region below the isthmus could be visualized.[143]

Isolation and culture of human follicular keratinocytes and dermal papilla (DP) cells

Excess skin from facelift procedures was collected with institutional review board (IRB) approval. Human hair follicles were isolated by microdissection. To isolate matrix cells and DP cells, the skin was cut at the dermosubcutaneous interface with a razor blade, hair follicles at anagen phase were selected, and matrix cell-

containing tissue fragments or DP cells were dissected out under the dissecting microscope. To isolate epithelial stem cells, the skin was incubated in dispase (Sigma,12.5 mg/ml in DMEM) overnight, each hair was pulled out of the skin, and hair follicles at telogen phase were selected and cut at the bulge region. Isolated tissue fragments were incubated in a mixture of 0.05% trypsin-EDTA (GIBCO) and Versene (0.53 mM EDTA 4Na, GIBCO) for 30 min at room temperature and spun down for 5 min at 800 rpm. The supernatant was removed, and isolated cells were plated on mitomycin C-treated (Sigma, 1.5 mg/ml DMEM for 2 h) 3T3-J2 cells in keratinocyte medium [KCM: DMEM and Ham's F12 (GIBCO, 4:1), adenine (Sigma, 180 mM), 10% fetal bovine serum (GIBCO), cholera toxin (ICN, 0.1 nM), penicillin/streptomycin (GIBCO, 100 U/ml and 100 mg/ml, respectively), hydrocortisone (Sigma, 0.4 mg/ml, 1.1 mM), T/T3 (transferrin, GIBCO, 5g/ml, 649 nM; and triiodo-L-thyronine, Sigma, 2 nM), insulin (Sigma, 5 mg/ml,862 nM), and EGF (Sigma, 10 ng/ml, 1.6 nM), pH 7.2] without Epidermal growth factor (EGF) and then changed to EGF-containing Keratinocyte culture medium (KCM). Cells were grown at 37°C in a humid atmosphere containing 5% CO2. All keratinocytes were fed with KCM containing EGF every 2 days and grown for 14-20 days. Isolated DP cells were seeded and grown without the feeder layer in Chang medium C (Irvine scientific) supplemented with 10% Fetal Bovine Serum (FBS) and penicillin/streptomycin. The cells maintained expression of smooth muscle actin throughout the culture process, indicating their preservation of the DP phenotype.[149]

Role of stem cells in alopecia

There is direct evidence in the mouse, and indirect evidence in humans, that compromising the integrity of the sebaceous gland and/or bulge is important in the development of alopecia.[150] The undifferentiated stem cell (SC) divides to generate transit amplifying (TA) cells that further differentiate into post-mitotic (PM) terminally differentiated cells that retain the SC phenotype in the 'SC-TA-PM' scheme initially proposed by Lavker and Sun.[151] The TA cells in turn undergo proliferation and terminal differentiation to populate the tissue.

Workers addressed the therapeutically relevant question of whether isolated hair follicle bulge epithelial cells can generate new hair follicles.[152,153] This became possible with the identification of bulge cell markers, CD34[154] and cytokeratin 15 promoter activity. It was shown that bulge cells do indeed retain their 'competence' for generating new hair follicles when removed from their environment or niche. This feat was only possible, when bulge cells were combined with 'inductive' dermal cells obtained from neonatal dermis.[155]

Although hair follicle bulge cells appear to be the only epithelial progenitors of the lower follicle in normal skin, this competence to form follicles is not exclusive to bulge cells. Interfollicular epidermal cells also retain some capacity to generate new hair follicles both in situ and after isolation; however, the efficiency of bulge cells to form follicles appears greater, suggesting that starting with a pure population of stem cells would increase the effectiveness of cell-based therapies. Additional studies suggest that other epithelia,[156] cultured epithelial cells,[157] corneal epithelium[158] or amnion[159] also have the capacity

to generate hair follicles when juxtaposed with inductive dermal cells. In fact, numerous types of stem cells, including embryonic, neural,[160] and mesenchymal bone marrow, all have the capacity to form skin and hair when introduced into blastocysts. This raises the intriguing possibility that these other types of stem cell could be coaxed into forming hair follicles by the proper inductive cells.[161]

Many investigators found that a follicular dermal papilla dissected from the base of an adult anagen hair follicle either fresh or after tissue culture expansion could induce new hair follicle formation in rodents if placed in proximity to the epithelium.[156,155,162-167] Perhaps the most dramatic demonstration of the inductive ability of the dermal portion of the follicle comes from the work of Reynolds et al transplanted the connective tissue sheath, a structure contiguous to the papilla, from the scalp of a man to the forearm of a woman. Remarkably, 3-5 weeks later new hair follicles were found at the site of implantation. The mesenchymal portion of the 'new' follicle arose from the donor male follicle, but the origin of the epithelial portion was not clear. An existing small follicle may have converted to a large hair follicle by the implanted sheath cells (morphogenetic switch), or the interfollicular epidermis in the recipient's arm may have generated a hair follicle de novo in response to the inductive dermal signals (neogensis model).[168]

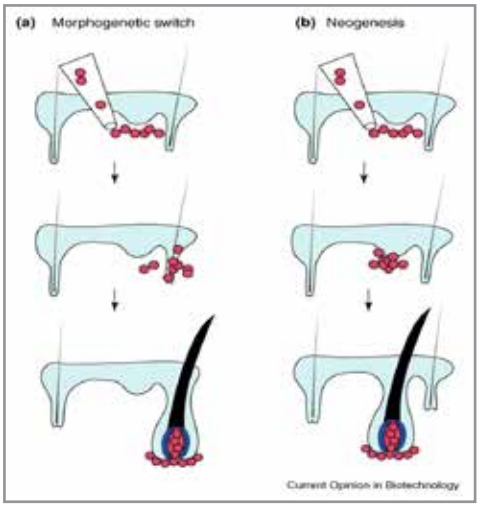

Fig. 3 (a) In the morphogenetic switch model.
(b) In the neogenesis model.[169]

For epithelial cells, Blanpain et al showed in mouse that cloned bulge cells can be amplified in culture using standard techniques with feeder cells, and then used in reconstitution assays to regenerate new hair follicles when combined with neonatal dermal cells. However, whether non-bulge keratinocytes possess similar properties was not reported. Future studies addressing these issues, especially in human systems, are necessary.[153]

For bioengineering the hair follicle, one could start with dermal elements from dissociated follicles with or without competent cells from the follicle or other epithelial sources. The number of dissociated cells would be expanded in culture and then dermal cells alone, or in combination with competent epithelial cells, re-introduced to the alopecic scalp. Previous studies have shown that starting with correctly placed inducer dermal cells will result in new follicle formation.[162,164,170] Moreover, starting with a combination of dissociated,[171] or aggregated, trichogenic epithelial and dermal cells has also proven to be an efficient way of producing new hair follicles.[172,173]

First attempts at cell-based approaches for treating alopecia are likely to use autologous tissue for bioengineering hair follicles to avoid immune rejection of the donor cells. However, the intriguing possibility that heterologous (allogeneic) hair follicle tissue could be developed for tissue transplantation exists, based on the concept that the hair follicle is an immune-privileged site

that does not express major histocompatibility complex (MHC) class I antigens.[152,174] As dermal hair follicle tissue has already been transplanted from one individual to another without evidence of rejection, this possibility may not be as implausible as originally thought. Nevertheless, the safety testing and regulatory hurdles for this type of approach would require enormous financial resources.[168]

Another possible approach for bioengineering hair follicles involves actually forming hair follicles as mini organs in vitro, and then transplanting the newly generated follicles back to the alopecic scalp. This sort of approach would require a much more complicated cell culture system involving three-dimensional matrices, perhaps embedded with appropriate growth factors, to allow both dermal and epidermal cells to differentiate towards a normal hair follicle. The bioengineering literature reports extensive experience with biocompatible materials that might be exploited here. The advantages to this approach include possible genetic manipulation of hair follicle cells ex vivo, and facilitation of the surgical placement of the new hair follicle in the proper orientation.[175,176]

Major challenges that need to be addressed with any type of cell-based treatment for alopecia include the efficiency of hair follicle formation and the choice of cell type. For example, how many new hair follicles can be generated from a given number of donated hair follicles? Clearly, the ratio of new hair follicles to donor hair follicles must be as high as possible to produce a clinically successful product. Other cell types, such as melanocytes and Merkel cells, normally reside in the follicle. Will these cells develop or be recruited to the new follicle? In addition, how can we be certain that the follicles formed will cycle? On this latter point, we have evidence that follicles formed from dissociated trichogenic cells will cycle repeatedly with the same phase as their follicles of origin.[177] Moreover, the follicles formed show the same morphology as the follicles from which the dermal cells were derived.[178]

An interesting aspect of new organ formation is raised by bone marrow transplant studies which show that cells arising from the bone marrow, which enter the circulation, may contribute to a wound or reparative cellular response.[179,180,181] It is notable that such cells will contribute to skin and hair follicle repair.[182] Would a regenerating system also attract cells that might affect (enhance or inhibit) organogenesis overall? Other challenges include how to achieve an appropriate patterning and angling of the hair follicle. Much progress has been made in understanding the molecular pathways activated during hair follicle embryogenesis and cycling.[183,184] Eventually, this understanding should lead to the generation of new pharmaceutical agents that specifically target these pathways.[185]

However, the complex timing and myriad gene expression changes required for orchestration of hair follicle development and cycling are likely to preclude a simple pharmaceutical approach to the treatment of advanced alopecia. By taking advantage of cell types that 'know' how to forma hair follicle, we assume cell-based therapies will arrive in the clinic sooner than the purely molecular approach. Although the time is ripe to successfully engineer new hair follicles, we recognize a lesson that workers in the fields of stem cell biology and biotechnology have painfully learned laboratory animal studies might not

translate to humans. Very few folliculoneogenesis studies have been conducted in humans. Our greatest successes have been made with animal models, and for hair growth (mammalian systems) that model has been the mouse. Intermediate studies could involve testing cell-based treatments on human skin grafted to immunodeficient mice. The ultimate test will be the clinical study.[186]

CONCLUSION

Alopecia areata is a psychologically debilitating disease process that has no cure and no uniformly dependable treatment. Corticosteroids (topical, local injections, and systemic) are the most popular, and reports support their efficacy in the treatment of AA. Other treatments that have been used with some success include: minoxidil, anthralin, DNCB, SADBE, PUVA, cyclosporine. With each treatment, side effects and cosmetically acceptable improvement must be considered.

The creation of new hair follicles for the treatment of alopecia through tissue engineering (stem cell) is achievable. The hair follicle reforms itself by means of interactions between competent epithelial stem cells and powerfully inductive dermal cells during its growth cycle. A product designed to form new hair follicles could be conceived to have the competent epithelial cells, the inductive dermal cells or a combination of both, delivered to the correct layer of the dermis.

The elements of hair follicle engineering, (stem cell) has efforts embrace the same engineering challenges that other organ systems will face, such as the eye, liver, pancreatic islet, and so on. Because of its inherent regenerative properties and the nature of the market demand, the hair follicle is likely to be the first organ regeneration system to successfully reach the clinic.

REFERENCES

1. Bergfeld, W. F. Hair disorders. In "Dermatology", 3rd ed., ed. by L. S. Moschlla and J. H. Hurley, W. B. Saunders Company, Philadelphia. 1992; pp. 1541-60.
2. Ohyama, M. Hair follicle bulge: a fascinating reservoir of epithelial stem cells. J. Dermatol. Sci. 2007; 46; 81-89.
3. Murphy, G. F. Histology of the skin. In "Lever's Histopathology of the Skin" 8th ed., ed. by D. Elder, R. Elenitsas, C. Jaworsky and B. Johnson Jr. Lippincott-Raven, Philadelphia, pp. 1997; 5-50.
4. Schneider, M. R., Schmidt-Ullrich, R. and Paus, R. The hair follicle as a dynamic miniorgan. Curr. Biol. 2009; 19; R132-R142.
5. Olsen EA. Hair, in Fitzpatrick's Dermatology in General Medicine. In: Freedberg IM. New York: McGraw-Hill. 2003:641-43.
6. Sehgal VN, Jain S. Alopecia areata: clinical perspective and an insight into pathogenesis. J Dermatol. 2003; 30:271-89.
7. Olsen EA, Hordinsky MK. Price VH, et al. Alopecia Areata Investigational Assessment Guidelines - Part II. J Am Acad Dermatol. 2004; 51:440-47.
8. Camacho F. Alopecia areata. Clinical characteristics and dermatopathology, in Trichology: diseases of the pilosebaceous follicle. Madrid, Aula Medical Group. 1997; S. A. 471-40.
9. Tan E, Tay YK, Goh CL, Chin Giam Y. The pattern and profile of alopecia areata in Singapore-a study of 219 Asians. Int J Dermatol. 2002; 41:748-53.
10. Bowen J. Two epidemics of alopecia areata in an asylum for girls. J Cutan General-Uri Dis. 1999; 17:399-404.
11. Stankler L. Synchronous alopecia in 2 siblings: a possible viral aetiology. Lancet. 1979; 1:1303-04.
12. Tosti A, La Placa M, Placucci F, et al. No correlation between CMV and alopecia areata. J Invest Dermatol. 1996; 107:443.
13. Plumbe S. A Practical Treatise on the Diseases of the Skin,4th edn. London: printed for Sherwood, Gilber and Piper, 1837.
14. Ruiz-Doblado S, Carrizosa A, Garcia-Hernandez MJ.

Alopecia areata: psychiatric comorbidity and adjustment to illness. Int J Dermatol. 2003; 42:434-37.
15. Yang S, Yang J, Liu JB, et al. The genetic epidemiology of alopecia areata in China. Br J Dermatol. 2004; 151:16-23.
16. Lenane P, Pope E, Krafchik B. Congenital alopecia areata.J Am Acad Dermatol. 2005; 52:S8-S11.
17. McDonagh AJ, Tazi-Ahnini R. Epidemiology and genetics of alopecia areata. Clin Exp Dermatol. 2002; 27:405-9.
18. Gilhar A, Kalish R. Alopecia areata: a tissue-specific autoimmune disease of the hair follicle. Autoimmunity Rev. 2006; 5:64-69.
19. Muller HK, Rook AJ, Kubba R. Immunohistology and autoantibody studies in alopecia areata. Br J Dermatol. 1980; 102:609-10.
20. Tan E, Tay YK, Giam YCh. A clinical study of childhood alopecia areata in Singapore. Pediatric Dermatol. 2002; 19:298-301.
21. Cunliffe WJ, Hall R, Newell DJ, et al. Vitiligo, thyroid disease and autoimmunity. Br J Dermatol. 1968; 80:135-39.
22. Wang SJ, Shohat T, Vadheim C, et al. Increased risk for type I (insulin-dependent) diabetes in relatives of patients with alopecia areata. Am J Med Genet. 1994; 51:234-39.
23. Werth VP, White WL, Sanchez MR, et al. Incidence of alopecia areata in lupus erythematosus. Arch Dermatol. 1992; 128:368-71.
24. Suzuki S, Shimoda M, Kawamura M, et al. Myasthenia gravis accompanied by alopecia areata: clinical and immunogenetic aspects. Eur J Neurol. 2005; 12:566-70.
25. Safavi KH, Muller SA, Suman VJ, et al. Incidence of alopecia areata in Olmsted County, Minnesota, 1975 through 1989. Mayo Clin Proc. 1995; 70:628-33
26. Hedstrand H, Perheentupa J, Ekwall O, et al. Antibodies against hair follicles are associated with alopecia totalis in autoimmune polyendocrine syndrome type I. J Invest Dermatol. 1999; 113:1054-58.
27. Corazza GR, Andreani ML, Venturo N, et al. Report of a new association. Gastroenterol. 1995; 109:1333-37.
28. Muller SA, Winkelmann RK. Alopecia areata. An evaluation of 736 patients. Arch Dermatol. 1963; 88:290-97.
29. Giovanni S. Recherches sur l histologie pathologique de la pelade. Ann Dermatol Syphiligr. 1991; 3:923-57.
30. Restrepo R, Mckee Ph, Calonje E. Diseases of the hair. In:Mckee Ph, Calonje E, Granter SR, eds. Pathology of theSkin with Clinical Correlations. 3th edn. Philadelphia: Elsevier Mosby. 2005:1073-79.
31. Ghersetich I, Campanile G, Loti T. Alopecia areata: immunohistochemistry and ultrastructure of infiltrate and identification of adhesion molecule receptors. Int J Dermatol. 1996; 35:28-33.
32. Hull SM, Nutbrown M, Pepall L, et al. Immunohistologic and ultrastructural comparison of the dermal papilla and hair follicle bulb from “active” and “normal” areas of alopecia areata. J Invest Dermatol. 1991; 96:673-81.
33. Igarashi R, Moroashi M, Takeuchi S. Immunofluorescence studies on complement components in the hair follicles of normal scalp and of scalp affected by alopecia areata. Acta Derm Venreol. 1981; 61:131-35.
34. Berker D, Baran D. RPR Handbook of Diseases of the Nails and Their Management. Oxford: Blackwell Science Ltd, 2004.
35. Cline DJ. Changes in hair color. Dermatol Clin. 1988; 6:295-303.
36. Shimmer BP, Parker KL. Goodman’s and Gillman’s: The Pharmacological Basis of Therapeutics. New York: McGraw-Hill. 2001:1661-63.
37. Dan Wasserman, Daniela Araucaria Guzman-Sanchez, Kimberly Scott, and Amy McMichael. Alopecia areata. Int J Dermatol. 2007; 46:121-31.
38. Garg S, Messenger AG. Alopecia areata: evidence-based treatments. Semin Cutan Med Surg. 2009; 28:15-8.
39. Ross EK, Shapiro J. Management of hair loss. Dermatol Clin. 2005; 23:227-43.
40. Madani S, Shapiro J. Alopecia areata update. J Am Acad Dermatol. 2000; 42:549-66.
41. Whiting DA. The Treatment Alopecia Areata Cutis. 1987; 40:247-50.
42. Price VH. Treatment of hair loss. New Engl J Med. 1999; 341:964-73.
43. Pascher F, Kurtin S, Andrade R. Assay of 0.2 percent fluocinolone acetonide cream for alopecia areata and totalis. efficacy side effects including histologic study

ensuing localized acneiform response. Dermatologica. 1970; 141:193-202.
44. Leyden JL, Kligman AM. Treatment of alopecia areata with steroid solution. Arch Dermatol. 1972; 106:924.
45. Cerottini JP, Panizzon RG, de Viragh PA. Multifocal alopecia areata during systemic cyclosporine A therapy. Dermatology. 1999; 198:415-17.
46. Montes LF. Topical halcinonide in alopecia areata and in alopecia totalis. J Cutan Pathol. 1977; 4:47-50.
47. Tosti A, Piraccini BM, Pazzaglia M, et al.Clobetasol propionate 0.05% under occlusion in the treatment of alopecia totalis/universalis. J Am Acad Dermatol. 2003; 49:96-98.
48. Bunker CB, Dowd PM. Alterations in scalp blood flow after the epicutaneous application of 3% minoxidil and 0.1% hexyl nicotinate in alopecia. Br J Dermatol. 1987; 117:668-69.
49. Lachgar S, Charveron M, Gall Y, Bonafe JL. Minoxidil upregulates the expression of vascular endothelial growth factor in human hair dermal papilla cells. Br J Dermatol. 1998; 138:407-11.
50. UnoH, Cappas A, Brigham P. Action of topicalminoxidil in the bald stump-tailed macaque. J Am Acad Dermatol. 1987; 16:657-68.
51. Buhl AE, Waldon DJ, Conrad SJ, Mulholland MJ, Shull KL, Kubicek MF, et al. Potassium channel conductance: a mechanism affecting hair growth both in vitro and in vivo. J Invest Dermatol. 1992; 98:315-19.
52. Galbraith GM, Thiers BH. In vitro suppression of human lymphocyte activity by minoxidil. Int J Dermatol. 1985; 24:249-51.
53. Price VH. Double-blind, placebo-controlled evaluation of topical minoxidil in extensive alopecia areata. J Am Acad Dermatol. 1987; 16:730-36.
54. Ranchoff RE, Bergfeld WF, Steck WD, et al. Extensive alopecia areata. Results of treatment with 3% top minoxidil cleve. Clin J Med. 1989; 56:149-54.
55. Fiedler VC, Wendrow A, Szpunar GJ, et al. Treatment resistant alopecia areata. Response to combination therapy with minoxidil plus anthralin. Arch Dermatol. 1990; 126:756-59.
56. Fiedler VC. Alopecia areata: current therapy. J Invest Dermatol. 1991; 96:69S.
57. Olsen EA, Dunlap FE, Funicella T, Koperski JA, Swinehart JM, Tschen EH, et al. A randomized clinical trial of 5% topical minoxidil versus 2% topical minoxidil and placebo in the treatment of androgenetic alopecia in men. J Am Acad Dermatol. 2002; 47:377-85.
58. Olsen EA, Whiting D, Bergfeld W, Miller J, Hordinsky M, WanserR, et al. A multicenter, randomized, placebo-controlled, double- blind clinical trial of a novel formulation of 5% minoxidil topical foam versus placebo in the treatment of androgenetic alopecia in men. J Am Acad Dermatol. 2007; 57:767-74.
59. Lucky AW, Piacquadio DJ, Ditre CM, Dunlap F, Kantor I, Pandya AG, et al. A randomized, placebo-controlled trial of 5% and 2% topical minoxidil solutions in the treatment of female pattern hair loss. J Am Acad Dermatol. 2004; 50:541-53.
60. Fiedler-Weiss VC, Buys CM. Response to minoxidil in severe alopecia areata correlates with T lymphocyte stimulation. Br J Dermatol. 1987; 117:759-63.
61. Fiedler-Weiss VC, Buys CM. Evaluation of anthralin in the treatment of alopecia areata. Arch Dermatol. 1987; 123:1491-93.
62. Schmoeckel C, Weissmann I, Plewig G, et al. Treatment of alopecia areata by anthralin-induced dermatitis. Arch Dermatol. 1979; 115:1254-55.
63. Nelson DA, Spielvogel RL. Anthralin therapy for alopecia areata. Int J Dermatol. 1985; 24:606-7.
64. McMichael AJ. Topical sensitizers in alopecia areata. Dermatol Nurs. 2004; 16:333-36.
65. Happle R. Antigenic competition as a therapeutic concept for alopecia areata. Arch Dermatol Res. 1980; 267:109-14.
66. Herbst V, Zoller M, Kissling S, Wenzel E, Stutz N, Freyschmidt-Paul P. Diphenylcyclopropenone treatment of alopecia areata induces apoptosis of perifollicular lymphocytes. Eur J Dermatol. 2006; 16:537-42.
67. Wasylyszyn T, Kozlowski W, Zabielski SL. Changes in distribution pattern of CD8 lymphocytes in the scalp in alopecia areata during treatment with diphencyprone. Arch Dermatol Res. 2007; 299:231-7.
68. Hoffmann R, Wenzel E, Huth A, van der Steen P, Schaufele M, Henninger HP, et al. Cytokine mRNA levels in Alopecia areata before and after treatment with the contact allergen diphenylcyclopropenone. J Invest Dermatol. 1994; 103:530-33.
69. Rosenberg EW, Drake L. In: Discussion of Dunaway

DA. Alopecia areata. Arch Dermatol. 1976; 112:256.
70. Strobel R, Rohrborn G. Mutagenic cell transforming activities 1-chlor-2, 4-dinitrobenzene (DNCB) squaricacid-dibutylester (SADBE) Arch Toxicol. 1980; 45:307-14.
71. Pardasani AG, Turner E, McMichael AJ. Squaric acid dibutylester: indications for use and efficacy in alopecia areata. Arch Dermatol. 2001; 137:970-72.
72. Wilkerson MG, Henkin J, Wilkin JK, et al. Squaric acid and esters: analysis for contaminants and stability in solvents. J Am Acad Dermatol. 1985; 13:229-34.
73. Breslow BM, Haynie R, Mirra J. Synthesis of diphencyclopropenone. J Am Chem Soc. 1959; 81:247-48.
74. Van der Steen PH, Happle R. Top immunotherapy alopecia areata. Dermatol Clin. 1993; 11:619-22.
75. Cotellessa C, Peris K, Caracciolo E, et al. The use of topical diphenylcyclopropenone for the treatment of extensive alopecia areata. J Am Acad Dermatol. 2001; 44:73-76.
76. McMichael AJ, Henderson RL Jr. Topical sensitizers in alopecia areata. Dermatol Nurs. 2004; 16:333-36.
77. Tosti A, De Padova MP, Minghetti G, et al. Therapies versus placebo in the treatment of patchy alopecia areata. J Am Acad Dermatol. 1986; 15:209-10.
78. Perret CM, Steijlen PM, Zaun H, et al. Erythema multiforme-like eruptions a rare side effect top immunotherapy with diphenylcyclopropenone Dermatologica. 1990; 180:5-7.
79. Van der Steen PH, Boezeman JB, Happle R. Topical immunotherapy for alopecia areata: re-evaluation of 139 cases after an additional follow-up period of 19 months. Dermatology. 1992; 184:198-201.
80. Higgins E, du Vivier A. Topical immunotherapy: unapproved uses, dosages or indications. Clin Dermatol. 2002, 515-21.
81. Duhra P, Foulds IS. Persistent vitiligo induced by diphencyprone. Br J Dermatol. 1990, 123:4 no 15-416.
82. Lawrence ID. Tacrolimus Dermatol Ther. 1998; 5: 74-84. (FK506): experience in dermatology.
83. Yamamoto S, Jiang H, Kato R. Stimulation of hair growth by topical application of FK 506, a potent immunosuppressive agent. J Invest Dermatol. 1994; 102:160-64.
84. McElwee KJ, Rushton DH, Trachy R, et al. Topical FK 506: a potent immunotherapy for alopecia areata? Studies using Dundee exp bald rat model. Br J Dermatol. 1997; 137:491-97.
85. Ehsani A, Toosi S, Seirafi H, Akhyani M, Hosseini M, Azadi R, et al. Capsaicin vs. clobetasol for the treatment of localized alopecia areata. J Eur Acad Dermatol Venereol 2009; Mar 5. [Epub ahead of print].
86. Hordinsky M, Ericson M. Autoimmunity: alopecia areata. J Investig Dermatol Symp Proc. 2004; 9:73-8.
87. Herane MI, Urbina F. Acquired trichomegaly of the eyelashes and hypertrichosis induced by bimatoprost. J Eur Acad Dermatol Venereol. 2004; 18:644-45.
88. Sasaki S, Hozumi Y, Kondo S. Influence of prostaglandin F2 alpha and its analogues on hair regrowth and follicular melanogenesis in a murine model. Exp Dermatol. 2005; 14:323-28.
89. Talpur R, Vu J, Bassett R, Stevens V, DuvicM. Phase I/II randomized bilateral half-head comparison of topical bexarotene 1% gel for alopecia areata. J Am Acad Dermatol. 2009; 61:592. e1-9.
90. Dillaha CJ, Rothman S. Therapeutic experiments in alopecia areata with orally administered cortisone. J Am Med Assoc. 1952; 150:546-50.
91. Burton JL, Shuster S. Large doses of glucocorticoid in the treatment of alopecia areata. Acta Derm Venereol. 1975; 55:493-96.
92. Olsen EA, Carson SC, Turney EA. Systemic steroids with or without 2% topical minoxidil in the treatment of alopecia areata. Arch Dermatol. 1992; 128:1467-73.
93. Friedli A, Labarthe MP, Engelhardt E, et al. Pulse methylprednisolone therapy for severe alopecia areata: an open prospective study of 45 patients. J Am Acad Dermatol. 1998; 39:597-602.
94. Assouly P, Reygagne P, Jouanique C, et al. Intravenous pulse methylprednisolone therapy for severe alopecia areata. An open study of 66 patients. Ann Dermatol Venereol. 2003; 130:326-30.
95. Thappa DM, Vijaikumar S. Intravenous dexamethasone pulse therapy for extensive alopecia areata. Indian J Dermatol. 1999; 44:187-90.
96. Winter RJ, Kern F, Blizzard RM. Prednisone therapy for alopecia areata. A follow-up report. Arch Dermatol. 1976; 112:1549-52.
97. Sharma VK. Pulsed administration of corticosteroids in the treatment of alopecia areata. Int J Dermatol.

1996; 35:133-36.
98. Sharma VK, Gupta S. Twice weekly 5 mg dexamethasone oral pulse in the treatment of extensive alopecia areata. J Dermatol. 1999; 26:562-65.
99. Kahan BD. Cyclosporine. N Engl J Med. 1989; 321:1725-38.
100. Taylor M, Ashcroft AT, Messenger AG. Cyclosporin A prolongs human hair growth in vitro. J Invest Dermatol. 1993; 100:237-39.
101. Gupta AK, Ellis CN, Nickoloff BJ, et al. Oral cyclosporine in the treatment of inflammatory and noninflammatory dermatoses. A clinical and immunopathologic analysis. Arch Dermatol. 1990; 126:339-50.
102. Ferrando J, Grimalt R. Partial response of severe alopecia areata to cyclosporine A. Dermatology. 1999; 199:67-69.
103. Ranganath VK, Furst DE. Disease-modifying antirheumatic drug use in the elderly rheumatoid arthritis patient. Rheum Dis Clin North Am. 2007; 33:197-217.
104. Ellis CN, Brown MF, Voorhees JJ. Sulfasalazine for alopecia areata. J Am Acad Dermatol. 2002; 46:541-44.
105. McMichael AJ. The new biologics in psoriasis: possible treatments for alopecia areata. J Invest Dermatol Symp Proc. 2003; 8:217-18.
106. Strober B, Siu K, Alexis A, et al. Etanercept does not effectively treat moderate to severe alopecia areata:an open-label study. J Am Acad Dermatol. 2005; 52:1082-84.
107. Posten W, Swan J. Recurrence of alopecia areata in a patient receiving etanercept injections. Arch Dermatol. 2005; 141:759-60.
108. Ettefagh L, Neodorost S, Mirmirani P. Alopecia areta in a patient using infliximab: new insights into the role of tumor necrosis factor on human hair follicles. Arch Dermatol. 2004; 140:1012.
109. Price VH, Hordinsky MK, Olsen EA, Roberts JL, Siegfried EC, Rafal ES, et al. Subcutaneous efalizumab is not effective in the treatment of alopecia areata. J Am Acad Dermatol. 2008; 58:395-402.
110. Pelivani N, Hassan AS, Braathen LR, Hunger RE, Yawalkar N. Alopecia areata universalis elicited during treatment with adalimumab. Dermatology. 2008; 216:320-23.
111. Fabre C, Dereure O. Worsening alopecia areata and de novo occurrence of multiple halo nevi in a patient receiving infliximab. Dermatology. 2008; 216:185-86.
112. Pan Y, Rao NA. Alopecia areata during etanercept therapy. Ocul Immunol Inflamm. 2009; 17:127-29.
113. Cipriani R, Perini GI, Rampinelli S. Paroxetine in alopecia areata. Int J Dermatol. 2001; 40:600-1.
114. Willemsen R, Vanderlinden J, Deconinck A, Roseeuw D. Hypnotherapeutic management of alopecia areata. J Am Acad Dermatol. 2006; 55:233-37.
115. Kamen D, Aranow C. Vitamin D in systemic lupus erythematosus. Curr Opin Rheumatol. 2008; 20:532-37.
116. Al-Mutairi N. 308-nm excimer laser for the treatment of alopecia areata. Dermatol Surg. 2007; 33:1483-87.
117. Zakaria W, Passeron T, Ostovari N, Lacour JP, Ortonne JP. 308-nm excimer laser therapy in alopecia areata. J Am Acad Dermatol. 2004; 51:837-38.
118. Porter D, Burton JL. A Comparison of intra-lesional triamcinolone hexacetonide and triamcinolone acetonide in alopecia areata. Br J Dermatol. 1971; 85:272-73.
119. Kurt S Stenn1 and George Cotsarelis. Bioengineering the hair follicle: fringe benefits of stem cell technology. Curr Opin Biotechnol. 2005; 16:1-5.
120. Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. Viable offspring derived from fetal and adult mammalian cells.Nature. 1997; 385:810-13.
121. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998; 282:1145-47.
122. Mansilla E, Marin GH, Sturla F, Drago HE, Gil MA, Salas E, et al. Human mesenchymal stem cells are tolerized by mice and improve skin and spinal cord injuries. Transplant Proc. 2005; 37:292-94.
123. Schultz SS. Adult stem cell application in spinal cord injury. Curr Drug Targets. 2005; 6:63-73.
124. Cotsarelis G, Sun TT, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell. 1990; 61:1329-37.
125. Nishimura EK, Granter SR, Fisher DE. Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche. Science 2005; 307:720-24.
126. Osawa M, Egawa G, Mak SS, Moriyama M, Freter R, Yonetani S, et al. Molecular characterization of melanocyte stem cells in their niche. Development. 2005; 132:5589-99.

127. Shi C, Zhu Y, Su Y, Cheng T. Stem cells and their applications in skin-cell therapy. Trends Biotechnol. 2006; 24:48-52.
128. Strem BM, Hicok KC, Zhu M, Wulur I, Alfonso Z, Schreiber RE, et al. Multipotential differentiation of adipose tissue-derived stem cells. Keio J Med. 2005; 54:132-41.
129. Scha ffler A, Bu chler C. Concise review: adipose tissue-derived stromal cellsebasic and clinical implications for novel cellbased therapies. Stem Cells. 2007; 25:818-27.
130. Freitas CS, Dalmau SR. Multiple sources of non-embryonic multipotent stem cells: processed lipoaspirates and dermis as promising alternatives to bone-marrow-derived cell therapies. Cell Tissue Res. 2006; 325:403-11.
131. Myung P, Ito M. Dissecting the bulge in hair regeneration. J Clin Invest. 2012; 122:448-54.
132. Cotsarelis G. The hair follicle: dying for attention. Am J Pathol. 1997; 151:1505-09.
133. Paus R. Control of the hair cycle and hair diseases as cycling disorders. Curr Opin Dermatol. 1996; 3:248-58.
134. Cotsarelis G. Epithelial stem cells: a folliculocentric view. J Invest Dermatol. 2006; 126:1459-68.
135. Kanitakis J, Bourchany D, Faure M, Claudy A. Expression of the hair stem cell-specific keratin 15 in pilar tumors of the skin. Eur J Dermatol. 1999; 9:363-65.
136. Akiyama M, Dale BA, Sun TT, Holbrook KA. Characterization of hair follicle bulge in human fetal skin: the human fetal bulge is a pool of undifferentiated keratinocytes. J Invest Dermatol. 1995; 105:844-50.
137. Cotsarelis G. Gene expression profiling gets to the root of human hair follicle stem cells. The Journal of Clinical Investigation. 2006; 16 (1):19-22.
138. Tiede S, Kloepper JE, Whiting DA, Paus R. The 'follicular trochanter': an epithelial compartment of the human hair follicle bulge region in need of further characterization. Br J Dermatol. 2007; 157:1013-16.
139. Paus R, Cotsarelis G. The biology of hair follicles. N Engl J Med. 1999; 341:491-97.
140. Ohyama M, Terunuma A, Tock CL. Characterization and isolation of stem cell-enriched human hair follicle bulge cells. J Clin Invest. 2006; 116:249-60.
141. Lyle S, Christofidou-Solomidou M, Liu Y et al. The C8/144B monoclonal antibody recognizes cytokeratin 15 and defines the location of human hair follicle stem cells. J Cell Sci. 1998; 111:3179-88.
142. Mobini N, Tam S, Kamino H. Possible role of the bulge region in the pathogenesis of inflammatory scarring alopecia: lichen planopilaris as the prototype. J Cutan Pathol. 2005; 32:675-79.
143. Hoang M. P., Keady M. and Mahalingam M. Stem cell markers (cytokeratin 15, CD34 and nestin) in primary scarring and nonscarring alopecia. Brit J Dermatol. 2009 (160):609-15.
144. Pozdnyakova O, Mahalingam M. Involvement of the bulge region in primary scarring alopecia. J Cutan Pathol. 2008; 35:922-25.
145. Al-Refu K, Edward S, Ingham E, Goodfield M. Expression of hair follicle stem cells detected by cytokeratin 15 stain: implications for pathogenesis of the scarring process in cutaneous lupus erythematosus. Br J Dermatol. 2009; 160:1188-96.
146. Poblet E, Jime´nez-Acosta F, Godı´nez JM et al. The immunohistochemical expression of CD3 in human hair follicle: a comparative study with bulge marker CK15. Clin Exp Dermatol. 2006; 31:807-12.
147. Nickoloff BJ. The human progenitor cell antigen (CD34) is localized on endothelial cells, dermal dendritic cells, and perifollicular cells in formalin-fixed normal skin, and on proliferating endothelial cells and stromal spindle-shaped cells in Kaposi's sarcoma. Arch Dermatol. 1991; 127:523-29.
148. Poblet E, Jimenez-Acosta F, Rocamora A. QBEND/10 (anti-CD34 antibody) in external root sheath cells and follicular tumors. J Cutan Pathol. 1994; 21:224-28.
149. Cecilia Roh, Qingfeng Tao, and Stephen Lyle.Dermal papilla-induced hair differentiation of adult epithelial stem cells from human skin. Physiol Genomic. 2004; 19:207-17.
150. Al-Refu K. Stem cells and alopecia: a review of pathogenesis. Brit J Dermatol. 2012 (167):479-84.
151. Lavker RM, Sun TT. Epidermal stem cells. J Invest Dermatol. 1983; 81: S121-7.
152. Morris RJ, Liu Y, Marles L, Yang Z, Trempus C, Li S, Lin JS, Sawicki JA, Cotsarelis G: Capturing and profiling adult hair follicle stem cells. Nat Biotechnol. 2004; 22:411-17.

153. Blanpain C, Lowry WE, Geoghegan A, Polak L, Fuchs E: Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. Cell. 2004; 118:635-48.
154. Trempus CS, Morris RJ, Bortner CD, Cotsarelis G, Faircloth RS, Reece JM, Tennant RW: Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface marker CD34. J Invest Dermatol. 2003; 120:501-11.
155. Liu Y, Lyle S, Yang Z, Cotsarelis G: Keratin 15 promoter targets putative epithelial stem cells in the hair follicle bulge. J Invest Dermatol. 2003; 121:963-68.
156. Ferraris C, Bernard BA, Dhouailly D: Adult epidermal keratinocytes are endowed with pilosebaceous forming abilities. Int J Dev Biol. 1997; 41:491-98.
157. Xing L, Kobayashi K: Ability of transplanted cultured epithelium to respond to dermal papillae. Tissue Eng. 2001; 7:535-44.
158. Ferraris C, Chaloin-Dufau C, Dhouailly D: Transdifferentiation of embryonic and postnatal rabbit corneal epithelial cells. Differentiation. 1994; 57:89-96.
159. Fliniaux I, Viallet JP, Dhouailly D, Jahoda CA: Transformation of amnion epithelium into skin and hair follicles. Differentiation. 2004; 72:558-65.
160. Clarke DL, Johansson CB, Wilbertz J, Veress B, Nilsson E, Karlstrom H, Lendahl U, Frisen J: Generalized potential of adult neural stem cells. Science. 2000; 288:1660-63.
161. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M et al.: Pluripotency of mesenchymal stem cells derived from adult marrow. Nature. 2002; 418:41-49.
162. Cohen J: The transplantation of individual rat and guinea-pig whisker papillae. J Embryol Exp Morphol. 1961; 9:117-27.
163. Jahoda CA, Horne KA, Oliver RF: Induction of hair growth by implantation of cultured dermal papilla cells. Nature. 1984; 311:560-62.
164. Kishimoto J, Burgeson RE, Morgan BA: Wnt signaling maintains the hair-inducing activity of the dermal papilla. Genes Dev. 2000; 14:1181-85.
165. McElwee KJ, Kissling S, Wenzel E, Huth A, Hoffmann R: Cultured peribulbar dermal sheath cells can induce hair follicle development and contribute to the dermal sheath and dermal papilla. J Invest Dermatol. 2003; 121:1267-75.
166. Oliver RF: Ectopic regeneration of whiskers in the hooded rat from implanted lengths of vibrissa follicle wall. J Embryol Exp Morphol. 1967; 17:27-34.
167. Robinson M, Reynolds AJ, Jahoda CA: Hair cycle stage of the mouse vibrissa follicle determines subsequent fiber growth and follicle behavior in vitro. J Invest Dermatol. 1997; 108:495-500.
168. Reynolds AJ, Lawrence C, Cserhalmi-Friedman PB, Christiano AM, Jahoda CA: Trans-gender induction of hair follicles. Nature. 1999; 402:33-34.
169. Stenn K.S. and Cotsarelis G. Bioengineering the hair follicle: fringe benefits of stem cell technology. Current Opinion in Biotechnology. 2005; 16:1-5.
170. Inamatsu M, Matsuzaki T, Iwanari H, Yoshizato K: Establishment of rat dermal papilla cell lines that sustain the potency to induce hair follicles from afollicular skin. J Invest Dermatol. 1998; 111:767-75.
171. Lichti U, Weinberg WC, Goodman L, Ledbetter S, Dooley T, Morgan D, Yuspa SH: In vivo regulation of murine hair growth:insights from grafting defined cell populations onto nude mice. J Invest Dermatol. 1993; 101:124S-29S.
172. Ihara S, Watanabe M, Nagao E, Shioya N: Formation of hair follicles from a single-cell suspension of embryonic rat skin by a two-step procedure in vitro. Cell Tissue Res. 1991; 266:65-73.
173. Layer PG, Robitzki A, Rothermel A, Willbold E: Of layers and spheres: the reaggregate approach in tissue engineering. Trends Neurosci. 2002; 25:131-34.
174. Paus R, Eichmuller S, Hofmann U, Czarnetzki BM, Robinson P: Expression of classical and nonclassicalMHCclass-I antigens in murine hair-follicles. Br J Dermatol. 1994; 0131:177-83.
175. Method of Tissue Engineering: Edited by Atala A, Lanza RP. London: Academic Press; 2002.
176. Ratner BD, Bryant SJ: Biomaterials: where we have been and where we are going. Annu Rev Biomed Eng. 2004; 6:41-75.
177. Zheng Y, Du X, Wang W, Boucher M, Parimoo S, Stenn K: Organogenesis from dissociated cells: generation of mature cycling hair follicles from skin-derived cells. J Invest Dermatol. 2005; 124:867-76.
178. Jahoda CA: Induction of follicle formation and hair

growth by vibrissa dermal papillae implanted into rat ear wounds: vibrissa-type fibres are specified. Development. 1992; 115:1103-09.

179. Borue X, Lee S, Grove J, Herzog EL, Harris R, Diflo T, Glusac E, Hyman K, Theise ND, Krause DS: Bone marrow-derived cells contribute to epithelial engraftment during wound healing. Am J Pathol. 2004; 65:1767-72.
180. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A: Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. Mol Med. 1994; 1:71-81.
181. Herzog EL, Chai L, Krause DS: Plasticity of marrow-derived stem cells. Blood. 2003; 102:3483-93.
182. Kataoka K, Medina RJ, Kageyama T, Miyazaki M, Yoshino T, Makino T, Huh NH: Participation of adult mouse bone marrow cells in reconstitution of skin. Am J Pathol. 2003; 163:1227-31.
183. Schmidt-Ullrich R, Paus R: Molecular principles of hair follicle induction and morphogenesis. Bioessays. 2005; 27:247-61.
184. Stenn KS, Paus R: Controls of hair follicle cycling. Physiol Rev. 2001; 81:449-94.
185. Cotsarelis G, Millar SE: Towards a molecular understanding of hair loss and its treatment. Trends Mol Med. 2001; 7:293-301.
186. Domashenko A, Gupta S, Cotsarelis G: Efficient delivery of transgenes to human hair follicle progenitor cells using topical lipoplex. Nat Biotechnol. 2000; 18:420-23.