Hair : Therapy & Transplantation

Review Article — **Open Access**

Stem Cells of the Hair Follicular Tissue: Application in Cell Based Therapy for Vitiligo

Anil Kumar[1,2], Sujata Mohanty[2*], Somesh Gupta[3] and Paulkhurana SM[1]

[1]Amity Institute of Biotechnology, AMITY University, Haryana, India
[2]Centre of Excellence for Stem Cell Research, AIIMS, New Delhi, India
[3]Department of Dermatology, AIIMS, New Delhi, India

Abstract

Stem cells have been found to reside in the different organs of the human body localized in specific niches. One such mini-organ is hair follicle wherein stem cells of epidermal origin have been reported. In the present review article we have reviewed in brief about the melanocyte stem cells of the hair follicle tissue, their identification, surface marker characteristics and role in the cell based therapy for vitiligo.

Keywords: Hair follicle; Stem cells; Mealnocyte; Transplantation; Cell therapy

Adult Stem Cells

A lot of evidence has been generated to testify that stem cells are not only an essential part of the embryo, but also, present in most organs of the mature adult body such as liver, intestine, bone marrow, eye, heart, kidney, skin and hair; to name a few of them. These organ-based stem cells, called adult stem cell (ASC) generate the progenitor cells for the periodic cell turnover of the organs' tissues. In addition, they are used for repair or replacement of cells that have been injured, destroyed or undergone apoptosis. Thus, whereas in the embryo, totipotent stem cells provide for the embryo's development, pluripotent stem cells based in mature organs are concerned with tissue homeostasis. The transition from totipotent embryonic stem cells to pluripotent organ based stem cells is not yet understood and it is unknown whether in the transition process, the embryonic stem cells lose some of their differentiation potential.

But the organ-based adult stem cells certainly retain their full ability of self-replication which is an essential component of 'stemness'. In most organs, adult stem cells form small cell aggregates generally located in one of the organ's relatively quiescent parts- the "stem cell niche". Asymmetric division, which yields one differentiating daughter cell and one self- replicated stem cell, occurs only to the extent that cell replacement is needed for periodic tissue turnover. In the case of tissue injury, however, this balanced system may be interrupted in order to rapidly provide stem cells and their progenitors needed for repair or replacement of the damaged tissue.

Hair Follicle Stem Cells

For many years, it was thought that the stem cells that regenerate hair follicles during the hair cycle are the highly proliferative matrix cells [1]. This model was later challenged when Montagna and Chase [2] observed that X-ray irradiation kills the matrix cells, but hairs can still re-form from cells within the outer root sheath cells (ORS). The ability of the upper ORS to act in concert with the dermal papilla (DP) to make hair follicles was further substantiated by dissection and transplantation experiments [3,4] Mathematical modeling supported the presence of stem cells which must be dividing at a slow pace to maintain the pool of progeny cells [5]. This notion was bolstered by administering repeated doses of marked nucleotide analogs such as BrdU to label the S-phase cells of the skin (pulse period) and then following the fate of the incorporated label over time (chase period). The differentiating cells are sloughed from the skin surface, and the more proliferative cells dilute their label as they divide, marking the least proliferative cells as label-retaining cells (LRCs) (Bickenbach 1981). The majority of LRCs in the skin resided in a specialized region at the base of the permanent segment of the hair follicle, known as the bulge. This is worth mentioning here that bulge was described more than a century ago, by histologist Stohr (Stohr 1903) (reviewed by Blanpain et al.) [6]. The bulge resides just below the sebaceous gland at a site where the arrector pili muscle attaches to the follicle.

Amongst the self-renewing compartments of mammalian epidermis, the hair follicle is an important component amongst others [7]. The hair follicle repeats its cyclic regeneration and regression with alternating phases, anagen (growing phase), catagen (regressing phase), and telogen (resting phase), to regrow new hair [8]. Hair follicle stem cells (HFSCs) are multipotent stem cells of the keratinocyte and melanocyte lineage that are located in the bulge area of each hair follicle [8-11] (Figure 1).

In addition to bulge, hair follicle houses stem cells in at least one more niches- the bulb. The DP in bulb of the hair follicle houses mesenchymal stem cells [12].

Localization of Stem Cell Niche in Hair Follicle

Several methods have been devised to identify epidermal stem cells and to try to demarcate them from other types of cell residing in the epidermis including transient amplifying cells and terminally differentiated cells [13,14]. The stem cells which are slow cycling in nature and retain the DNA label such as tritiatedthymidine or bromodeoxyuridine for a longer chase period (4-10 weeks), during which the label is lost, from rapidly proliferating cells, such as, the transient amplifying cells as a consequence of proliferation-associated dilution, while the rarely dividing stem cells retain the label for prolonged periods and are therefore called label-retaining cells. The second method makes use of the high proliferative capacity of epidermal stem cells. Using this method the proliferative potential of cultured cells is assessed by examining the clonogenicity of individual
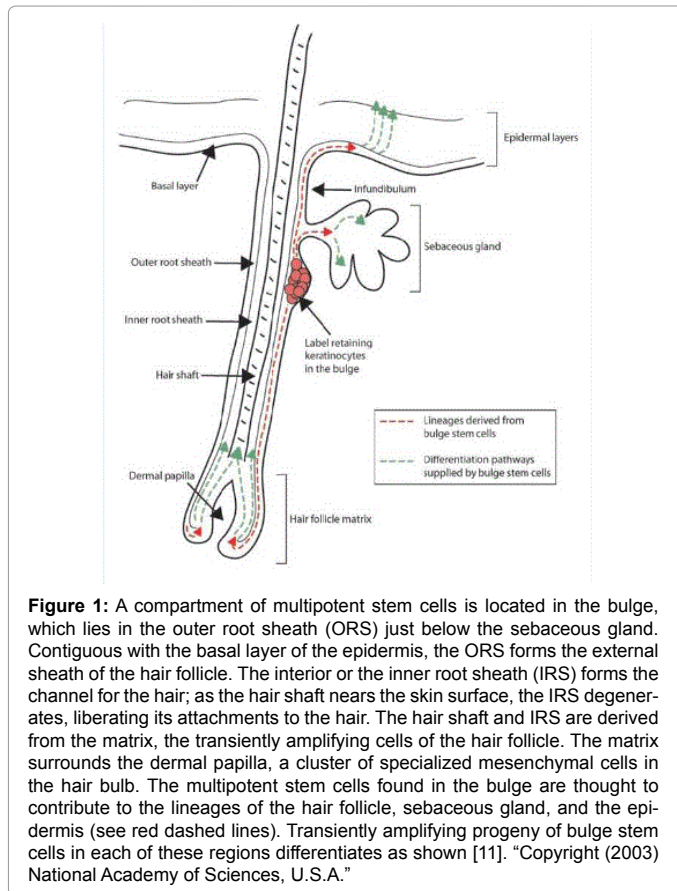
Figure 1: A compartment of multipotent stem cells is located in the bulge, which lies in the outer root sheath (ORS) just below the sebaceous gland. Contiguous with the basal layer of the epidermis, the ORS forms the external sheath of the hair follicle. The interior or the inner root sheath (IRS) forms the channel for the hair; as the hair shaft nears the skin surface, the IRS degenerates, liberating its attachments to the hair. The hair shaft and IRS are derived from the matrix, the transiently amplifying cells of the hair follicle. The matrix surrounds the dermal papilla, a cluster of specialized mesenchymal cells in the hair bulb. The multipotent stem cells found in the bulge are thought to contribute to the lineages of the hair follicle, sebaceous gland, and the epidermis (see red dashed lines). Transiently amplifying progeny of bulge stem cells in each of these regions differentiates as shown [11]. "Copyright (2003) National Academy of Sciences, U.S.A."

cells through serial passage or colony-forming efficacy (CFE). Although these two methods help in the identification of epidermal stem cells, they do not allow for the easy isolation of living stem cells for further analysis. Several epidermal stem cell markers have been identified during the past few years through the use of a candidate approach or, more recently, by global gene expression profiling. However, reliable and specific stem cell markers for epidermal stem cells and their transient amplifying cell progeny are still lacking. Therefore, rather than relying upon a single stem cell marker, a panel of markers have been identified to isolate the epidermal stem cells [9,15-17].

Stem cells of epithelia reside in niches, such as the deep epidermal ridges or the bulge of the hair follicle, where they are insulated from physical and mechanical influences [18]. These stem cells produce specific cytokeratins, such as K15 and K19 [18,19]. Originally, all basal cells in stratified epithelia were thought to be able to produce new cells to maintain the epidermis because mitotic figures were observed in the entire basal layer of the fetal human epidermis, in which all basal cells produce the K15, which is characteristic of epidermal stem cells [20]. However, the concepts of epidermal stem cells, transient amplifying cells and epidermal proliferative units were developed by Alonso and Fuchs [11] when it was recognized that the telomeres of chromosomes limit the number of possible cell divisions to about 30 within the lifetime of a cell lineage and that this number of epidermal cell divisions would not suffice to compensate for the loss of cells through normal desquamation and shedding [21]. Under the new concept, stem cells have a special capacity to renew themselves over the lifetime of an organism, and they divide into two different daughter cells, namely one stem cell and one transient amplifying cell [11,18,20]. Primary cell cultures of stem cells from hair follicle and the epidermis

generate a variety of cell types, namely stem cells (10%), transient amplifying cells (40%) and keratinocytes (50%), with each cell type having different potentials to proliferate and generate new cell clones [22]. In the inter follicular epidermis, 2-5% of the basal cells are stem cells and produce K15 [20]. Drawing upon the observations by Alonso and Fuchs [11], the new model of epidermal renewal proposes that the basal layer of stratified epithelia is comprised not only of stem cells and transient amplifying cells but also of cells that have already started their differentiation into keratinocytes. The transient amplifying cells replicate themselves four to eight times before they finally divide into keratinocytes. The descendants of one stem cell (i.e. the transient amplifying cells and keratinocytes) are collectively called an 'epidermal proliferative unit' (EPU) [23].

Isolation of Enriched Hair Bulge Cell Population

Investigation of bulge cell biology and the use of bulge cells for clinical applications such as regenerative medicine or gene therapy require the isolation of living bulge cells [24,25]. Morphology based manual microdissection has been used to isolate bulge cells from hair follicles [25,26], however, this technique is time-consuming, requires skill and the purity of collected sample is unclear. The identification of bulge cell specific markers enables a more accurate and high-throughput isolation of those cells and makes further investigation feasible. Lyle et al. first reported that K15 is preferentially expressed in the bulge cells in human hair follicles [27]. Antibodies against K15 preferentially stain the outermost layer of the ORS around the insertion point of the arrector pili muscle, where the label-retaining cells were detected [27]. Since low-level expression of K15 can be detected in the lower follicle, K15 is not a marker with a 'high-enough specificity' for human bulge cells [9,24]. Microarray analyses identified a panel of cell surface markers for human bulge cells. CD200 and CD59 are up-regulated in bulge compared to other defined hair follicle regions, while CD24, CD34, CD71 and CD146 is down-regulated. Particularly, CD200 is preferentially distributed in bulge area and represents the best positive surface marker for the human bulge cells. Thus, living human bulge cells could be isolated as $CD200^{hi}24lo34lo71lo146lo$ cells, using the combination of positive and negative markers. Furthermore, $CD200^{hi}24^{lo}34^{lo}71^{lo}146^{lo}$ bulge cells isolated from human hair follicles demonstrate a high proliferative capacity *in vitro* suggesting successful enrichment of stem cells was possible using these markers. Other markers to identify hair follicle bulge stem cells include frizzled homolog 1 that is up-regulated in human bulge cells [9]. Some up-regulated genes in human bulge cells [9] deserve further investigation for their possible clinical applications. For example, CD200, a newly identified marker for human bulge cells, has been reported to play an important role in the regulation of immune response. CD200 is a type-1 transmembrane glycoprotein that delivers an inhibitory immunoregulatory signal through the CD200 receptors on immune cells [28]. CD200 depleted mouse skin is highly susceptible to perifollicular inflammation resulting in alopecia, suggesting that enhanced expression of CD200 within the human bulge region may provide a degree of immune privilege to stem cells [24,28]. Over-expression of CD200 in the bulge area or inhibition of CD200-CD200 receptor signaling might be a useful approach to protect the bulge stem cells from inflammatory destruction in scarring alopecias [29]. The clinical relevance of the follicle bulge area is not limited to disorders of keratinocyte lineage.

Melanocyte Stem Cells of the Hair Follicle

The cellular reservoirs for epidermal and hair follicle melanocytes were originally considered to be distinct populations. In human, the former has been referred to as 'amelanotic melanocytes.' In 1956,

Montagna and Chase [2] reported the existence of a distinct cell population that does not contain visible melanin pigment but can be distinguished morphologically from surrounding keratinocytes in the outer root sheath (ORS) of human hair follicles 3 weeks following ionizing irradiation. In the clinic, skin repigmentation, in vitiligo, an acquired pigmentary disorder, usually begins at the orifices of hair follicles, then enlarges, and coalesces to cover the entire depigmented skin area. This phenomenon is called 'follicular repigmentation' and is characterized by repigmentation of an unpigmented skin area starting from the hair follicle orifices, suggesting the existence of a melanocyte 'reservoir' population in the skin [30,31]. Starricco studied the phenomenon extensively and found that the amelanotic ORS cell population, which does not contain any melanin pigment and is negative for the dopa reaction, contains nuclei that stain densely with toluidine blue and thionine [32]. Starricco subsequently found that this population can be activated after excision of the epidermis or by UV therapy [31]. He assumed that the amelanotic melanocyte population provides DOPA-positive dendritic melanocytes that migrate into, and fill, the epidermis for follicular repigmentation. However, these amelanotic melanocytes were not always clearly distinguished from Langerhans progenitors because of the lack of specific lineage markers available at the time [31,33]. Ortonne et al. examined the unpigmented skin of vitiligo patients after treatment with UV therapy using scanning electron microscopy and DOPA staining. These authors found that the pigmented islands that appeared after the treatment had follicular cores containing melanocytes with large cell bodies that could be distinguished from other cells in the ORS. They also postulated that there is a melanocyte reservoir in human hair follicles [30]. Other studies also supported the view that ORS melanocytes are able to proliferate and to provide progeny to the epidermis in response to specific stimuli, such as UV exposure and epidermal wound healing [34]. Subsequently, the existence of melanocyte lineage cells with significant potential to proliferate was shown by Tobin et al. [35] with in vitro cultures of human hair follicles. In 1996, Horikawa et al. reported that cells that stain with NK1beteb, a monoclonal antibody that recognizes the premelanosomal protein SILV/GP100/PMEL17, are distributed in the ORS at the mid-portion of anagen hair follicles and are quite similar to 'amelanotic melanocytes' in their morphology and distribution [36]. Narisawa et al. [37] found that amelanotic melanocytes stained by NK1beteb possess limited numbers of dendrites and are distributed abundantly in the bulge area of human hair follicles. This series of studies demonstrated the lineage identity of 'amelanotic melanocytes' in human hair follicles and led to the view that 'amelanotic melanocytes' (melanoblasts) in the ORS represent a reservoir that can replenish melanocytes in the epidermis when necessary.

However, no obvious spatially restricted niche of melanocyte stem cells has been found in glabrous areas, which are abundant in melanocytes. Melanocyte stem cells in hair follicles lose their self-renewal capacity with aging, leading to hair graying. By contrast, skin melanocytes sustain lifelong proliferative potential. Although it is a rare case, repopulation of melanocytes occurs in vitiliginous areas after UVA treatment, even in glabrous skin [38]. These data suggest that extrafollicular melanocyte stem cells might exist, and could potentially function as a reservoir for melanocytes in postnatal epidermis [39-41].

Vitiligo and Its Management by Surgery

Vitiligo is a disorder characterized by the loss of epidermal melanocytes leading to severe psychological problems among the patients. The exact etiology of this disease is not established and several reasons proposed include stress, genetic susceptibility, autoimmunity,

melatonin receptor dysfunction, and impaired melanocyte migration and/or proliferation.

Both non-surgical and surgical therapies have been employed in the management of vitiligo. Surgical therapies are useful when disease is recalcitrant to medical therapies and stable for at least one year. Tissue grafts can treat only a small area; therefore cellular grafts, like basal cell layer enriched epidermal suspension are preferred over tissue grafts when large area is to be treated. Tissue grafting procedures include transplantation of mini grafts, thin and ultra-thin split thickness grafts (STSG) and suction blister epidermal grafts (SBEG). Cellular grafting techniques include transplantation of non-cultured basal cell enriched epidermal suspension (NCES) and cultured melanocytes (CM) or cultured epidermis (CE) [42-47]. The major advantage of non-cultured cell suspension and culture expanded cell suspension is that they permit the treatment of the affected area many folds larger than the donor area [48-50]. However, culture techniques employed for the expansion of the cell number are time consuming, expensive and also require trained manpower and well-equipped tissue culture laboratory. In addition, there are concerns about the safety of the techniques owing to the xenobiotic properties of some of the additives in the culture medium [51].

These limitations of culture expanded cells were sorted out with the introduction of the non-cultured cellular grafting techniques in 1992 by Gauthier and Surleve-Bazeille [52] and followed by several other vitiligo surgery groups. Non-cultured cell transplant can cover larger vitiliginous areas (8-to10-fold size of donor skin) and the procedure can be completed in few hours on an outpatient basis. In summary, cultured cell transplants are technically more challenging and expensive than non-cultured epidermal cell suspension; hence the latter provides a simple, effective and aesthetically more suitable treatment option [43,44,48,52-58]

Hair Follicle Melanocytes and their Role in Vitiligo Repigmentation

Role of hair follicle in the pigmentation of the vitiliginous lesion has been well proved in the landmark paper by Cui et al. [34]. In conjunction with previous reports by Starrico [32,59,60], they observed that some DOPA-negative "inactive" melanocytes (proposed to be melanocyte stem cells) were found to reside in the outer root sheath and bulge areas of the hair follicle. These melanocytes did not produce melanin under normal conditions but became active to produce melanin when stimulated either by ultraviolet radiation or dermabrasion. This was a landmark observation which proposed the hair follicle to be the reservoir of "inactive" melanocytes (or the so called melanocyte stem cells). It was also observed that in vitiliginous lesions, there was destruction of only the active (DOPA-positive) melanocytes while the inactive melanocytes in the ORS of the hair follicle were undamaged. It was these melanocytes that were proposed to lead to repigmentation in vitiligo by dividing and migrating upward along the surface of hair follicle to the nearby epidermis where they continued to move radially and lead to repigmentation of surrounding skin as observed by Cui et al. [34]. The finding that not only did melanocyte stem cells reside in hair follicles but could also in addition, migrate to surrounding skin was another landmark observation.

These findings suggested that inactive melanocytes in the ORS of the hair follicle divide proliferate and mature during the process of repigmentation in the vitiligo and may be potentially harvested and cultivated for therapeutic purposes in vitiligo. The findings of the Cui et al. [34] have been proved by several groups and the inactive melanocytes are now taken as melanocyte stem cells [24,61-71].

Transplantation of Hair Follicle ORS Cells Suspension in Vitiligo

In a quest to further concentrate pigment cell population in the non-cultured cell suspension for transplantation in vitiligo, the major breakthrough came with the paper of Vaenscheidt and Hunziker [72], in a small case series, they have used single cell suspension of 'plucked' hair follicles in the treatment of vitiligo. They found almost complete (>90%) repigmentation in 3 of 5 patients with vitiligo, around 50% repigmentation in one patient and less than 10% repigmentation in one patient. Their technique is simple, non-invasive and allows easy, immediate and repeated application. However the cell yield is less in case of plucked hair follicles and optimization of cell harvest from the hair follicular unit needs to be standardized for optimum yield.

Our group has devised a very innovative method of harvesting the follicular tissue by the FUE method for cell based therapy in vitiligo. The ORS cells suspension of the hair follicles obtained by FUE method was used for autologous transplantation in vitiligo patients. This method resulted in significant repigmentation in the majority of the patients [73].

Future Direction

Although many questions remain to be answered, it is clear that because of their plasticity and accessibility, hair follicle stem cells provide a major resource for therapeutic applications in tissue repair and regeneration. The methodology to culture stem cells has opened-up its application in several areas of research and therapeutic applications. Their fantastic growth capacity can be successfully exploited to produce epithelial cells that may be transplanted to treat extensive autologous deep burn wounds and pigment disorders [74]. In addition to their multi-potency, stem cells can be genetically engineered using viral vector constructs [75], which allows for usage in gene therapy. Reprogramming the cells i.e. generation of induced pluripotent stem cells (iPSCs), represents an ideal cell source for future cell therapy and regenerative medicine [76,77]. Recently, Novak et al. have described a detailed and reproducible method to derive iPSCs from plucked human hair follicle keratinocytes [78], to make the derivation of iPSC from a noninvasive tissue source.

The recent advances in biotechnology, such as genomics, proteomics, *in vivo* epidermal targeting, green fluorescent protein (GFP) labeling and adeno-viral and retro-viral gene transfers, will allow for much better understanding of stem cell biology. These novel technological approaches will allow the identification of specific molecular markers which will aid further in the isolation of stem cells, thus, opening unlimited possibilities for therapeutic approaches.

References

1. Kligman AM (1959) The human hair cycle. J Invest Dermatol 33: 307-316.

2. Montagna W, Chase HB (1956) Histology and cytochemistry of human skin. X. X-irradiation of the scalp. Am J Anat 99: 415-445.

3. Jahoda CA, Horne KA, Oliver RF (1984) Induction of hair growth by implantation of cultured dermal papilla cells. Nature 311: 560-562.

4. Oliver RF (1966) Whisker growth after removal of the dermal papilla and lengths of follicle in the hooded rat. J Embryol Exp Morphol 15: 331-347.

5. Potten C, Wichmann HE, Loeffler M, Dobek K, Major D (1982) Evidence for discrete cell kinetic subpopulations in mouse epidermis based on mathematical analysis. Cell Tissue Kinet 15: 305-329.

6. Blanpain C, Fuchs E (2006) Epidermal stem cells of the skin. Annu Rev Cell Dev Biol 22: 339-373.

7. Gude D (2011) Hair follicle stem cells: a new arena. Int J Trichology 3: 125-126.

8. Fuchs E (2007) Scratching the surface of skin development. Nature 445: 834-842.

9. Cotsarelis G (2006) Epithelial stem cells: a folliculocentric view. J Invest Dermatol 126: 1459-1468.

10. Xia L, Liu Q, Zhang W, Zhou G, Cao Y, et al. (2012) Enhanced proliferation and functions of in vitro expanded human hair follicle outer root sheath cells by low oxygen tension culture. Tissue Eng Part C Methods 18: 603-613.

11. Alonso L, Fuchs E (2003) Stem cells of the skin epithelium. Proc Natl Acad Sci U S A 100 Suppl 1: 11830-11835.

12. Sellheyer K, Krah D (2010) Cutaneous mesenchymal stem cells: status of current knowledge, implications for dermatopathology. J Cutan Pathol 37: 624-634.

13. Tiede S, Kloepper JE, Bodò E, Tiwari S, Kruse C, et al. (2007) Hair follicle stem cells: walking the maze. Eur J Cell Biol 86: 355-376.

14. Kaur P (2006) Interfollicular epidermal stem cells: identification, challenges, potential. J Invest Dermatol 126: 1450-1458.

15. Braun KM, Watt FM (2004) Epidermal label-retaining cells: background and recent applications. J Investig Dermatol Symp Proc 9: 196-201.

16. Cotsarelis G, Sun TT, Lavker RM (1990) Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell 61: 1329-1337.

17. Cotsarelis G, Cheng SZ, Dong G, Sun TT, Lavker RM (1989) Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. Cell 57: 201-209.

18. Watt FM (1998) Epidermal stem cells: markers, patterning and the control of stem cell fate. Philos Trans R Soc Lond B Biol Sci 353: 831-837.

19. Narisawa Y, Hashimoto K, Kohda H (1994) Immunohistochemical demonstration of keratin 19 expression in isolated human hair follicles. J Invest Dermatol 103: 191-195.

20. Webb A, Li A, Kaur P (2004) Location and phenotype of human adult keratinocyte stem cells of the skin.72: 387-395.

21. Harley CB (1991) Telomere loss: mitotic clock or genetic time bomb? Mutat Res 256: 271-282.

22. Papini S, Cecchetti D, Campani D, Fitzgerald W, Grivel JC, et al. (2003) Isolation and clonal analysis of human epidermal keratinocyte stem cells in long-term culture. Stem Cells 21: 481-494.

23. Strachan LR, Ghadially R (2008) Tiers of clonal organization in the epidermis: the epidermal proliferation unit revisited. Stem Cell Rev 4: 149-157.

24. Ohyama M, Terunuma A, Tock CL, Radonovich MF, Pise-Masison CA, et al. (2006) Characterization and isolation of stem cell-enriched human hair follicle bulge cells. J Clin Invest 116: 249-260.

25. Kobayashi K, Rochat A, Barrandon Y (1993) Segregation of keratinocyte colony-forming cells in the bulge of the rat vibrissa. Proc Natl Acad Sci U S A 90: 7391-7395.

26. Rochat A, Kobayashi K, Barrandon Y (1994) Location of stem cells of human hair follicles by clonal analysis. Cell 76: 1063-1073.

27. Lyle S, Christofidou-Solomidou M, Liu Y, Elder DE, Albelda S, et al. (1998) The C8/144B monoclonal antibody recognizes cytokeratin 15 and defines the location of human hair follicle stem cells. J Cell Sci 111: 3179-3188.

28. Rosenblum MD, Yancey KB, Olasz EB, Truitt RL (2006) CD200, a "no danger" signal for hair follicles. J Dermatol Sci 41: 165-174.

29. Mobini N, Tam S, Kamino H (2005) Possible role of the bulge region in the pathogenesis of inflammatory scarring alopecia: lichen planopilaris as the prototype. J Cutan Pathol 32: 675-679.

30. Ortonne JP, Schmitt D, Thivolet J (1980) PUVA-induced repigmentation of vitiligo: scanning electron microscopy of hair follicles. J Invest Dermatol 74: 40-42.

31. STARICCO RG (1963) Amelanotic melanocytes in the outer sheath of the human hair follicle and their role in the repigmentation of regenerated epidermis. Ann N Y Acad Sci 100: 239-255.

32. STARICCO RG (1959) Amelanotic melanocytes in the outer sheath of the human hair follicle. J Invest Dermatol 33: 295-297.

33. Shukla RC (1969) The origin of Langerhans' cell from the melanocyte. Acta Anat (Basel) 73: 372-388.

34. Cui J1, Shen LY, Wang GC (1991) Role of hair follicles in the repigmentation of vitiligo. J Invest Dermatol 97: 410-416.

35. Tobin DJ, Colen SR, Bystryn JC (1995) Isolation and long-term culture of human hair-follicle melanocytes. J Invest Dermatol 104: 86-89.

36. Horikawa T, Norris DA, Johnson TW, Zekman T, Dunscomb N, et al. (1996) DOPA-negative melanocytes in the outer root sheath of human hair follicles express premelanosomal antigens but not a melanosomal antigen or the melanosome-associated glycoproteins tyrosinase, TRP-1, and TRP-2. J Invest Dermatol 106: 28-35.

37. Narisawa Y, Kohda H, Tanaka T (1997) Three-dimensional demonstration of melanocyte distribution of human hair follicles: special reference to the bulge area. Acta Derm Venereol 77: 97-101.

38. Davids LM, du Toit E, Kidson SH, Todd G (2009) A rare repigmentation pattern in a vitiligo patient: a clue to an epidermal stem-cell reservoir of melanocytes? Clin Exp Dermatol 34: 246-248.

39. Li L, Fukunaga-Kalabis M, Yu H, Xu X, Kong J, et al. (2010) Human dermal stem cells differentiate into functional epidermal melanocytes. J Cell Sci 123: 853-860.

40. Li L, Fukunaga-Kalabis M, Herlyn M (2012) Isolation and cultivation of dermal stem cells that differentiate into functional epidermal melanocytes. Methods Mol Biol 806: 15-29.

41. Tobin DJ, Paus R (2001) Graying: gerontobiology of the hair follicle pigmentary unit. Exp Gerontol 36: 29-54.

42. Bahadoran P (2007) Classification of surgical therapies for vitiligo. In Surgical Management of Vitiligo 59 -68.

43. Gupta S, Shroff S, Gupta S (1999) Modified technique of suction blistering for epidermal grafting in vitiligo. Int J Dermatol 38: 306-309.

44. Gupta S, Goel A, Kanwar AJ, Kumar B (2006) Autologous melanocyte transfer via epidermal grafts for lip vitiligo. Int J Dermatol 45: 747-750.

45. Falabella R (2005) Surgical approaches for stable vitiligo. Dermatol Surg 31: 1277-1284.

46. Löntz W, Olsson MJ, Moellmann G, Lerner AB (1994) Pigment cell transplantation for treatment of vitiligo: a progress report. J Am Acad Dermatol 30: 591-597.

47. Ongenae K, Van Geel N, Naeyaert JM (2001) Autologous cellular suspensions and sheets in the treatment of achromic disorders: the need for future controlled studies. Dermatology 202: 158-161.

48. Olsson MJ, Juhlin L (1993) Repigmentation of vitiligo by transplantation of cultured autologous melanocytes. Acta Derm Venereol 73: 49-51.

49. Redondo P, Del Olmo J, García-Guzman M, Guembe L, Prósper F (2008) Repigmentation of vitiligo by transplantation of autologous melanocyte cells cultured on amniotic membrane. Br J Dermatol 158: 1168-1171.

50. Pianigiani E, Risulo M, Andreassi A, Taddeucci P, Ierardi F, et al. (2005) Autologous epidermal cultures and narrow-band ultraviolet B in the surgical treatment of vitiligo. Dermatol Surg 31: 155-159.

51. Van Geel N, Goh BK, Wallaeys E, De Keyser S, Lambert J (2011) A Review of Non-cultured Epidermal Cellular Grafting in Vitiligo. J Cutan Aesthet Surg 4: 17-22.

52. Gauthier Y, Surleve-Bazeille JE (1992) Autologous grafting with noncultured melanocytes: a simplified method for treatment of depigmented lesions. J Am Acad Dermatol 26: 191-194.

53. Gupta S, Jain VK, Saraswat PK (1999) Suction blister epidermal grafting versus punch skin grafting in recalcitrant and stable vitiligo. Dermatol Surg 25: 955-958.

54. Czajkowski R (2004) Comparison of melanocytes transplantation methods for the treatment of vitiligo. Dermatol Surg 30: 1400-1405.

55. Chen YF, Chang JS, Yang PY, Hung CM, Huang MH, et al. (2000) Transplant of cultured autologous pure melanocytes after laser-abrasion for the treatment of segmental vitiligo. J Dermatol 27: 434-439.

56. Chen YF, Yang PY, Hung CM, Hu DN (2001) Transplantation of autologous cultured melanocytes for treatment of large segmental vitiligo. J Am Acad Dermatol 44: 543-545.

57. Jha AK, Pandey SS, Gulati AK, Bansal V, Shukla VK, et al. (1993) Inoculation of a cultured autologous epidermal suspension containing melanocytes in vitiligo. Arch Dermatol 129: 785-786.

58. Falabella R, Escobar C, Borrero I (1989) Transplantation of in vitro-cultured epidermis bearing melanocytes for repigmenting vitiligo. J Am Acad Dermatol 21: 257-264.

59. Staricco RG, Miller-Milinska A (1962) Activation of the amelanotic melanocytes in the outer root sheath of the hair follicle following ultra violet rays exposure. J Invest Dermatol 39: 163-164.

60. Staricco R (1961) Mechanism of migration of melanocytes from the hair follicle into the epidermis following dermabrasion. J Invest Dermatol 36: 99-104.

61. Nishimura EK, Granter SR, Fisher DE (2005) Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche. Science 307: 720-724.

62. Slominski A, Wortsman J, Plonka PM, Schallreuter KU, Paus R, et al. (2005) Hair follicle pigmentation. J Invest Dermatol 124: 13-21.

63. Tobin DJ (2011) The cell biology of human hair follicle pigmentation. Pigment Cell Melanoma Res 24: 75-88.

64. Tobin DJ (2009) Aging of the hair follicle pigmentation system. Int J Trichology 1: 83-93.

65. Lavker RM, Sun TT, Oshima H, Barrandon Y, Akiyama M, et al. (2003) Hair follicle stem cells. J Investig Dermatol Symp Proc 8: 28-38.

66. Levy V, Lindon C, Harfe BD, Morgan BA (2005) Distinct stem cell populations regenerate the follicle and interfollicular epidermis. Dev Cell 9: 855-861.

67. Lavker RM, Miller S, Wilson C, Cotsarelis G, Wei ZG, et al. (1993) Hair follicle stem cells: their location, role in hair cycle, and involvement in skin tumor formation. J Invest Dermatol 101: 16S-26S.

68. Zhu WY, Zhang RZ, Ma HJ, Wang DG (2004) Isolation and culture of amelanotic melanocytes from human hair follicles. Pigment Cell Res 17: 668-673.

69. Nishimura EK (2011) Melanocyte stem cells: a melanocyte reservoir in hair follicles for hair and skin pigmentation. Pigment Cell Melanoma Res 24: 401-410.

70. Nishimura EK, Jordan SA, Oshima H, Yoshida H, Osawa M, et al. (2002) Dominant role of the niche in melanocyte stem-cell fate determination. Nature 416: 854-860.

71. Tobin DJ (2011) The cell biology of human hair follicle pigmentation. Pigment Cell Melanoma Res 24: 75-88.

72. Vanscheidt W, Hunziker T (2009) Repigmentation by outer-root-sheath-derived melanocytes: proof of concept in vitiligo and leucoderma. Dermatology 218: 342-343.

73. Mohanty S, Kumar A, Dhawan J, Sreenivas V, Gupta S (2011) Noncultured extracted hair follicle outer root sheath cell suspension for transplantation in vitiligo. Br J Dermatol 164: 1241-1246.

74. Gallico GG et al. (1984) Permanent coverage of large burn wounds with autologous cultured human epithelium. N Engl J Med 311: 448-51.

75. Ghazizadeh S, Taichman LB (2001) Multiple classes of stem cells in cutaneous epithelium: a lineage analysis of adult mouse skin. EMBO J 20: 1215-1222.

76. Takahashi K, Tanabe K, Ohnuki M (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors 131: 861-872.

77. Lowry WE, Richter L, Yachechko R, Pyle AD, Tchieu J, et al. (2008) Generation of human induced pluripotent stem cells from dermal fibroblasts. Proc Natl Acad Sci U S A 105: 2883-2888.

78. Novak A, Shtrichman R, Germanguz I, Segev H, Levin ZN (2010) Enhanced Reprogramming and Cardiac Differentiation of Human Keratinocytes Derived from Plucked Hair Follicles, Using a Single Excisable Lentivirus. Cellular Reprogramming.