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Review of hair follicle dermal cells

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Abstract

Hair follicle stem cells in the epithelial bulge are responsible for the continual regeneration of the hair follicle during cycling. The bulge cells reside in a niche composed of dermal cells. The dermal compartment of the hair follicle consists of the dermal papilla and dermal sheath. Interactions between hair follicle epithelial and dermal cells are necessary for hair follicle morphogenesis during development and in hair reconstitution assays. Dermal papilla and dermal sheath cells express specific markers and possess distinctive morphology and behavior in culture. These cells can induce hair follicle differentiation in epithelial cells and are required in hair reconstitution assays either in the form of intact tissue, dissociated freshly-prepared cells or cultured cells. This review will focus on hair follicle dermal cells since most therapeutic efforts to date have concentrated on this aspect of the hair follicle, with the idea that enriching hair-inductive dermal cell populations and expanding their number by culture while maintaining their properties, will establish an efficient hair reconstitution assay that could eventually have therapeutic implications.

Keywords

Hair follicle; Dermal papilla; Dermal sheath; Reconstitution assay

1. Dermal components of the hair follicle

1.1. Introduction

The hair follicle is composed of epidermal (epithelial) and dermal (mesenchymal) compartments and their interaction plays an important role in the morphogenesis and growth of the hair follicle [1,2]. Effective cross-talk between these two compartments is also thought to be key for successful reconstitution of hair follicles for research or therapeutic purposes. Generally dermal cells are considered as inducers and epithelial cells as responders in the process of hair formation although the signaling in between the two cells type is reciprocal and complicated.

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Several models have been established for the study of dermal-epidermal interactions, as well as the reconstitution of hair follicles [3]. Most hair reconstitution assays take place in vivo in immunodeficient host mice. Although these assays work well with mouse cells, regenerating human hair follicles is still a challenge requiring breakthroughs in several aspects, including enriching cells with trichogenic capacity, maintaining their trichogenic capacity during processing, and providing them with an adequate host environment.

This article focuses on the dermal components of the hair follicle and provides an overview on the role of dermal cells in hair reconstitution assays, which hold promise for dissecting out factors and cellular subpopulations necessary for hair follicle regeneration.

1.2. Dermal papilla (DP) and dermal sheath (DS)

The dermal portion of the hair follicle can be divided into two compartments, the dermal papilla (DP) and dermal sheath (DS) [4] (Fig. 1). The DP is located at the base of the hair follicle. The DS, or connective tissue sheath, lines the epithelium of the hair follicle from the bulge level downward and is contiguous with the base of the DP through a stalk. DP and DS are separated from the epithelial portion of the hair follicle by a basement membrane. The DS consists of three layers of collagen fibers running in different directions, with fibroblasts mostly residing in the thickened middle collagen layer [5]. Cells within DP and DS are specialized fibroblasts of mesenchymal origin. However, expression of neuronal markers by DP cells and their transcription profiles suggest a neural crest origin [6–8]. By tracing the progeny of neural-crest stem cells, investigators found that a portion of the DP cells are derived from the neural crest [6].

DS has been considered as a cellular reservoir of DP cells during the hair follicle cycle[9], and it was hypothesized that stem cells might reside in the DS similar to its epithelial counterpart, the outer root sheath [10]. DS cells share similar characteristics with the DP and can regenerate a new DP after loss of the DP [11–13]. In classic transection studies of rat vibrissae, Oliver and colleagues demonstrated that removal of the lower follicle resulted in regeneration of the DP, apparently from the DS, while removal of the follicle at the level of the bulge did not allow for DP regeneration [14]. There may be two-way cellular traffic between the DP and DS during normal hair follicle cycling [15]. The DP reaches its maximal size in anagen IV when cell number is double that in telogen. This increase in cell number is thought to be due to recruitment of cells from the DS. DP cells may migrate out into the DS again prior to the next resting phase [9]. One recent study suggests that thrombin signaling through PI3K-Akt pathway regulates the transformation between DS and DP cells [16]. The exact relationship between the DP and DS awaits specific promoters that target each cell population. This approach has been useful for understanding heterogeneity of epidermal stem cells [10].

2. Dermal cells and dermal signals in hair morphogenesis and cycling

2.1. Dermal cells in hair morphogenesis and cycling

During embryonic hair follicle development, mesenchymal cells aggregate immediately below the epidermis in a patterned manner. These aggregates or “condensates” mark the location of the new hair follicle. The mesenchymal condensates maintain close contact with the base of down-growing epithelial columns and are eventually ensheathed by the hair bulb during the peg stage [17]. In a fully developed anagen hair follicle, the DP resides deep in the subcutaneous fat and is surrounded by hair matrix cells. In catagen, the DP moves up to the dermis as the epithelial strand regresses. As the secondary hair germ forms from the bottom of the bulge at the end of catagen, the DP comes to rest immediately adjacent to these cells that will form the next lower hair follicle. [18].

2.2. Selected dermal signals during hair follicle morphogenesis

This topic has been reviewed extensively [2,8,19] and here we mention only some major players. Wnt signaling in the dermis may be the "first dermal message" responsible for inducing formation of hair placodes from the overlying epidermis [2]. Dickkopf-1 (Dkk-1), a potent inhibitor of Wnt signaling is expressed by the interfollicular dermis surrounding new hair follicles at this stage [20]. Other dermal factors required for epithelial down-growth during hair morphogenesis include fibroblast growth factors and the bone morphogenetic protein (BMP) inhibitor, noggin [21,22].

Shh signaling is important for early DP development and maturation. It is expressed by the hair follicle epithelium and influences both mesenchyme and epithelium. In the Shh knockout mouse, hair follicle development is severely compromised; either arrested in the germ stage or exhibiting tiny hair follicle structures expressing hair shaft differentiation keratins [23–25]. Recent studies show primary cilia in DP cells are required for Shh signaling, and laminin-511 is critical for the formation of primary cilia in DP, as well as maintaining the expression of noggin [26,27]. PDGF-A is another epidermal factor which cooperates with Shh signaling and contributes to the formation of DP and DS [25].

3. Isolation and culture of dermal cells

3.1. Techniques for isolating and culturing dermal cells

The most widely used method to isolate DP is surgical micro-dissection, which has been well established in rat and murine vibrissae follicles as well as human hair follicles [28,29]. Surgical micro-dissection has also been used to isolate DS adjacent to DP [11,12]. However, the technique is laborious and time-consuming. Outgrowths of cells from DP explants can be expected within 1 week in culture medium. Cultured DP cells show flattened and polygonal morphology and tend to grow into multi-layered aggregates in the first few passages. The behavior of aggregate formation in DP cell culture seems to be correlated with their hair inductivity [30]. DP cells gradually lost their proliferative capacity after being passaged. Adding FGF-2 into the culture medium promotes long-term culture of mouse vibrissa and human DP cells to more than 30 passages [30].

Human DP can be harvested more efficiently by applying dispase and subsequent collagenase treatment to the lower anagen hair follicles which reside in the subcutaneous fat [31]. Taking advantage of transgenic expression of fluorescent protein in targeted cells and fluorescence activated cell sorting (FACS), dissociated DP cells can be obtained in a larger scale in mice using *versican* or *Lef1* promotors which are preferentially up-regulated in DP [8,32].

3.2. Dermal-epidermal interaction in vitro

Although DP cells and keratinocytes change their properties in culture, co-culturing these two cells in vivo still provides useful information about their interactions. Keratinocytes co-cultured with DP cells have increased proliferation rate and show significant migration toward DP cells as well as conditioned medium prepared with cultured DP cells [33]. When matrix cells from the hair bulb grow on the top of DP cells, occasionally keratinocytic spheres form and are surrounded by DP cells with the formation of basement membrane-like structure. This phenomenon, which showed the attempt to form hair follicles in vitro, was only observed when combining matrix cells with DP cells.

4. Biochemical and molecular signatures of dermal cells

Cells within DP and DS are specialized mesenchymal cells and express specific enzymes and molecules. Although the functions of most marker proteins are unknown, they have been

widely used to identify DP and DS. The expression of some markers, e.g. alkaline phosphatase and versican correlates with hair inductive properties.

4.1. Alkaline phosphatase (AP)

The activity of AP has been used as a marker to detect the presence of DP and regarded as an indicator for hair inductivity [13,34]. Handjiski et al. show that pelage DP of mice expressed strong and persistent AP activity throughout the entire hair cycle [35]. However, a recent study by Iida et al. shows dynamic change of AP activity in DP and bulbar dermal sheath. AP activity in DP reach its maximal level in early anagen, and decreased at the proximal half (below Auber's line) of DP after mid-anagen growing phase [34]. In DS, AP activity is shown in proximal DS adjacent to DP, with the highest level detected in early anagen [13,34]. The hair-inductivity of cultured DP cells is known to decrease after passage, as is the expression of AP [36]. The temporal and spatial changes of AP activity coincide with the hair-inductive property of DP and DS.

4.2. α-Smooth muscle actin (αSMA)

αSMA was present in the mid- to lower DS in rat and human hair follicles but not in DP [37]. However, DP cells become αSMA-positive in culture [37]. Therefore, αSMA is a marker for DS in vivo, and a marker for both DP and DS in vitro.

4.3. Versican

In human hair follicles, versican is reported specifically expressed in DP during anagen. Weak versican immunoreactivity has also been shown at the dermal sheath outside K15-positive bulge epithelial cells. Versican expression in DP is lost in miniaturized hair follicles of androgenetic alopecia [38]. In mouse, versican is expressed in anagen hair follicles but absent in telogen hair follicles. Therefore, versican may play an important role in anagen induction and maintenance of anagen. Ascorbic acid 2-phosphate induces expression of versican in human dermal cells which may in turn enhance the initiation and growth of hair follicles [39]. Kishimoto et al. use GFP driven by versican promoter as a way to enrich DP cells by FACS [32]. These GFP-positive cells show behavior and morphology consistent with DP cells. They induce hair neogenesis in engraft assay when combined with epidermal cells, while GFP-negative cells did not induce the formation of hair follicles [32]. Subsequent studies showed that while Versican-GFP cells are enriched for DP, more specific DP markers were needed.

4.4. Corin

Corin encodes a transmembrane protease that is expressed in the heart and participates in the processing of natriuretic peptides. In mouse pelage skin, Corin is expressed specifically in the DP from the earliest stage [40]. Corin also plays a role in the coat color specification. However, it not required for hair morphogenesis based on lack of phenotype by mutation of *Corin* gene in mice [40].

4.5. CD133

CD133, or Prominin-1, is a known hematopoietic stem cell marker that is strongly expressed in DP of stage 3–4 developing hair follicles and early anagen in mouse skin. CD133-positive cells isolated from mouse skin by FACS resemble DP cells in morphology and behavior when plated in culture and they have similar molecular and transcript profiles [41]. CD133-positive dermal cells, but not CD133-negative dermal cells, induce hair follicle neogenesis 10 times more efficiently than unsorted dermal cells in terms of required cell numbers [41]. The different result by CD133-positive and negative cells demonstrates the power of surface markers in isolating subpopulations from hair follicles and the importance of choosing the right subpopulation for hair regeneration assay.

5. Dermal cells in hair reconstitution assays and maintenance of hair inductivity

The hair inductive property of dermal cells, especially DP cells, has been clearly demonstrated. The epithelial part of a pre-existing hair follicle needs a DP to maintain its growth, and dissociated epidermal cells require the guidance of dermal cells to be organized into complicated hair structures, otherwise, a simple epithelial cyst forms. Therefore, dermal cells are critical in hair regeneration assay and it is important to keep dermal cells inductive during preparation.

5.1. Hair inductivity in dermal cells

The inductive property in dermal cells was elegantly shown by Oliver when he transposed DP beneath the upper half of amputated vibrissa hair follicles [42]. He and his colleagues then transplanted DP into afollicular skin and induced hair growth [43]. Cultured DP and DS cells, as well as intact DS tissue are able to reconstitute a new functioning DP in vivo [11–13,44]. Reynolds et al. introduced DS from male human scalp into the forearm skin of a female donor and induced growth of a terminal hair follicle with a DP containing a Y-chromosome indicating the donor origin [12]. Although we cannot be sure of the target epithelial population in this experiment, a subsequent study also showed trans-species induction of hair follicles by human DP [45]. If validated, these studies are significant for their demonstration of DP inductive abilities as well as the possible immune privilege state of the hair follicle championed by Paus and coworkers [46].

The inductive property of dermal cells is affected by donor status including hair cycle stage and age. Both the hair-forming ability and the hair-inductivity of DP change during hair cycle and affect the frequency of hair formation in transplantation study [47]. Dermal condensates from embryonic tissue and DP from adult skin induce hair formation through different mechanism [48]. When combining glabrous skin from adult mice onto embryonic dermis with dermal condensates, hair neogenesis follows the process of embryonic hair development. When using adult DP from vibrissa to induce hair neogenesis from the same epidermis, hair placode-like structures are not found.

The inductive property seems to be uniformly distributed in cells in a single DP although cells in different area express different markers. Osada et al. showed 80% clones from single DP cells have hair-inducing ability [49]. Each individual DP is designated to induce different type of hair follicles. Sox2-positive DP cells only induce guard/awl/auchen follicles while the DP of a zigzag hair follicle is Sox2-negative [7].

5.2. Hair reconstitution assays: in vivo model

Hair reconstitution assays are useful for assessing the hair inductive properties of isolated cell populations. Early reconstitution assays consisted of transposing dermal tissue or cells beneath or in close contact to the upper half of an amputated hair follicle in vivo [42] (Fig. 2). The inserted dermal tissue regenerates a new DP and helps to restore the truncated epithelial structure with new hair shaft growth. This type of model restores a pre-existing injured hair follicle rather than regenerating a hair follicle *de novo*. It is also laborious to dissect and amputate hair follicles and insert dermal population. Only large follicles can be used in this model so there are many limitations in its application. In the subsequent studies, dermal tissues or cells are implanted into the dermis of the skin in order to induce the formation of hair follicles from the overlying epidermis [43,50]. The origin of the dermal tissue determines the type of new hair follicles [51]. Questions still exist in some studies whether these implanted dermal tissues or cells induce de novo hair follicles, or transform pre-existing hair follicles into another

type. Hair formation by implantation of DP cells into glabrous skin favors de novo hair neogenesis [48,50].

More recently, two hair regeneration models are established using dissociated cell aggregates or single cells from hair follicles as the epidermal and dermal components (Fig. 3). This type of model gives us more flexibility to select and manipulate epidermal and dermal populations. The first model involves the use of a silicon graft chamber [52]. The mixture of epidermal and dermal cells is placed onto a full thickness wound on the back of immunodeficient mice. The wound is covered by a bell-shaped silicon chamber which confines and protects the cells on the wound bed. The chamber is usually taken off after 1 week and hair neogenesis can be seen from the surface of back skin in 3 weeks by using cells from mouse neonates [53].

In the second method, the mixture of epidermal and dermal cells is injected subcutaneously into the back skin of immunodeficient mice [54]. The injected epidermal cells aggregate into large cystic spheres seen as nodules in the host skin. Stenn calls this the “patch assay” [55]. Epidermal aggregates undergo central apoptosis and result in cyst formation. Dermal condensates outside the epidermal spheres start to induce hair follicles growing outward from the cyst wall with hair shafts projecting inward to the cyst cavity [55]. For epidermal and dermal cells from mouse neonates, this process of hair neogenesis takes less than 2 weeks. The back skin is cut off and the patch with regenerated hair follicles can be seen at the undersurface of the skin (Fig. 4).

The two models have been used widely. We injected a mixture of K15-positive epithelial cells from adult mice and dermal cells from mouse neonates into immunodeficient mice. Isolated adult epithelial stem cells from the hair follicle were multipotent and capable of regenerating the cutaneous epithelial lineages of the hair follicle, sebaceous gland and interfollicular epidermis. This study highlighted the importance of choosing the epithelial cell subpopulation with stem cell characteristics to reconstitute hair follicles [54]. Similarly, Ito et al. use CD133 as a marker to enrich hair-inductive dermal cells for hair regeneration assay.

In another in vivo model, glabrous skin was treated by dispase to create a small pocket between epidermis and dermis. Dermal cells are then inserted into the pocket before the whole skin grafted into nude mice [48,49].

However, the use of immunodeficient host mice in these in vivo models possesses drawbacks especially when applying these systems to regenerate human hair follicles. Very little is known about the influence of host factors on hair follicle neogenesis. Yet host immunity is not totally ablated and may confound the result. The placement of human cells into mouse hosts further aggravates this issue. These host factors may explain the low efficiency of these in vivo hair regeneration models in which several thousands to millions of cells are required to regenerate a single hair follicle. It may also explain why regeneration of pure human hair follicles has never been achieved so far.

5.3. In vitro hair regeneration model

In vitro environment is more controllable with fewer unknown factors. An in vitro hair reconstitution assay can provide high throughput analysis and production. However, it is not easy to simulate the complicated niche where hair follicles reside. Usually collagen is used as the matrix for dermal-epidermal interaction to take place. Epidermal and dermal cells can either be incorporated into the gel or sit on the top of the gel [56]. Dermal-epidermal interaction can also take place in a hanging-drop system [57]. However, all the structures that initially formed in the gel or liquid phase in vitro have to be eventually grafted into immunodeficient host mice in order to achieve complete hair morphogenesis [57,58]. Otherwise, only cell aggregates can

be seen which are still far from the structure of a real hair follicle. This suggests that current in vitro systems are still over-simplified for complete hair morphogenesis.

5.4. Regeneration of human hair follicles

Current success in de novo hair regeneration has been dependent on the use of mouse cells, especially cells from newborn or embryonic mice. Ehama et al. used a graft chamber to generate hair follicle-like structure from the combination of human epidermal cells and mouse dermal cells [53]. No pure human hair follicles can be made so far, suggesting that factors or microenvironment are missing.

6. Maintaining hair inductivity in dermal cell culture

It is important to keep cells competent in hair regeneration assays while processing and expanding them in culture. However, it is known that cultured DP cells gradually lose their hair inductivity and proliferative capacity after being passaged [30,59].

6.1. Sphere formation

Only early passaged DP cells manifesting aggregative behavior are able to form DP after implantation into transected upper hair follicles [59]. Cultured DP cells grown as spheres showed higher hair-inductivity than 2-D cells and these spheres retain their hair inductivity even in later passage (passage 26) [30].

In order to enhance self-aggregating and spheroid-forming behavior, DP cells can be cultured on a biomaterial surface with lower adhesivity [60]. However, the growth rate of DP cells becomes compromised on this surface. In addition, fibronectin coating on culture dish enhanced the number of spheroids by 67% [61].

6.2. BMP signaling

Hair bulb and DP are rich for BMPs and BMP receptor 1a. When DP cells are cultured in medium containing BMP 2, 4, 5 or 6, their expression of AP is preserved relative to cells cultured without BMPs [36]. BMP6-treated mouse DP cells have improved hair inductivity compared to control DP cells and the inductive capacity is retained up until passage 8. By ablating BMP receptor 1a in cultured DP cells, DP cells fail to reconstitute hair follicles [36].

6.3. Wnt Signaling

Activation of Wnt signaling is important for the initiation and maintenance of hair morphogenesis [2]. Kishimoto et al. demonstrate that Wnt-3a treated DP cells had a higher capacity to induce hair formation compared to non-treated or Shh-treated DP cells in engraft study [62]. Similar result from Ouji et al. showed that co-transplantation of Wnt-10b-secreting cells promoted hair neogenesis [63].

Glycogen synthase kinase-3 (GSK-3) inhibits Wnt signaling by phosphorylating β-catenin. GSK-3 inhibitor-treated human DP cells showed increased activity and expression of AP and IGF-1, two indicators of hair-inductivity. However, the hair regeneration assay using GSK-3-treated human DP cells with mouse epidermal cells fails to result in hair neogenesis [64].

7. Dermal cells in hair diseases

The role of the DP in diseases of skin and hair is not known, but some have speculated that androgenetic alopecia, which is characterized by miniaturized hair follicles and shortening of anagen phase in a defined pattern, may be due to the effect of testosterone and dihydrotestosterone acting on androgen receptors in the DP and causing changes in

transcription of genes such as TGF-β and IGF-1 [65–67]. Androgens may also drive the DP to secret inhibitory autocrine factors [68]. Bahta et al. cultured DP cell from balding and non-balding scalp and found that balding DP cells showed characteristics of senescence including loss of proliferation capacity, change of morphology and expression of senescence-related markers and oxidative stress markers such as $p16^{INK4a}$/pRb, heat shock protein-27 [69]. One recent study showed androgen regulated Wnt signaling in DP cells again suggesting their involvement in the disease process of AGA [66].

8. Conclusion

Dermal cells play a pivotal role in the regulation of hair growth. For hair follicle regeneration purposes, important issues include isolation of inductive dermal population, expanding their number by culture, maintenance of their hair inductive property and providing them an adequate niche and exogenous signals to enhance their interaction with epidermal cells toward the fate of hair neogenesis. An ideal hair regeneration model for human hair follicles has yet to be developed. The efficiency has to be improved with fewer starting cell number and higher number of regenerated hair follicles. The variables should be easily controllable for studying hair biology. The multipotent cells reside in dermal compartment of the hair follicle warrant further studies for their potential clinical application in regenerative medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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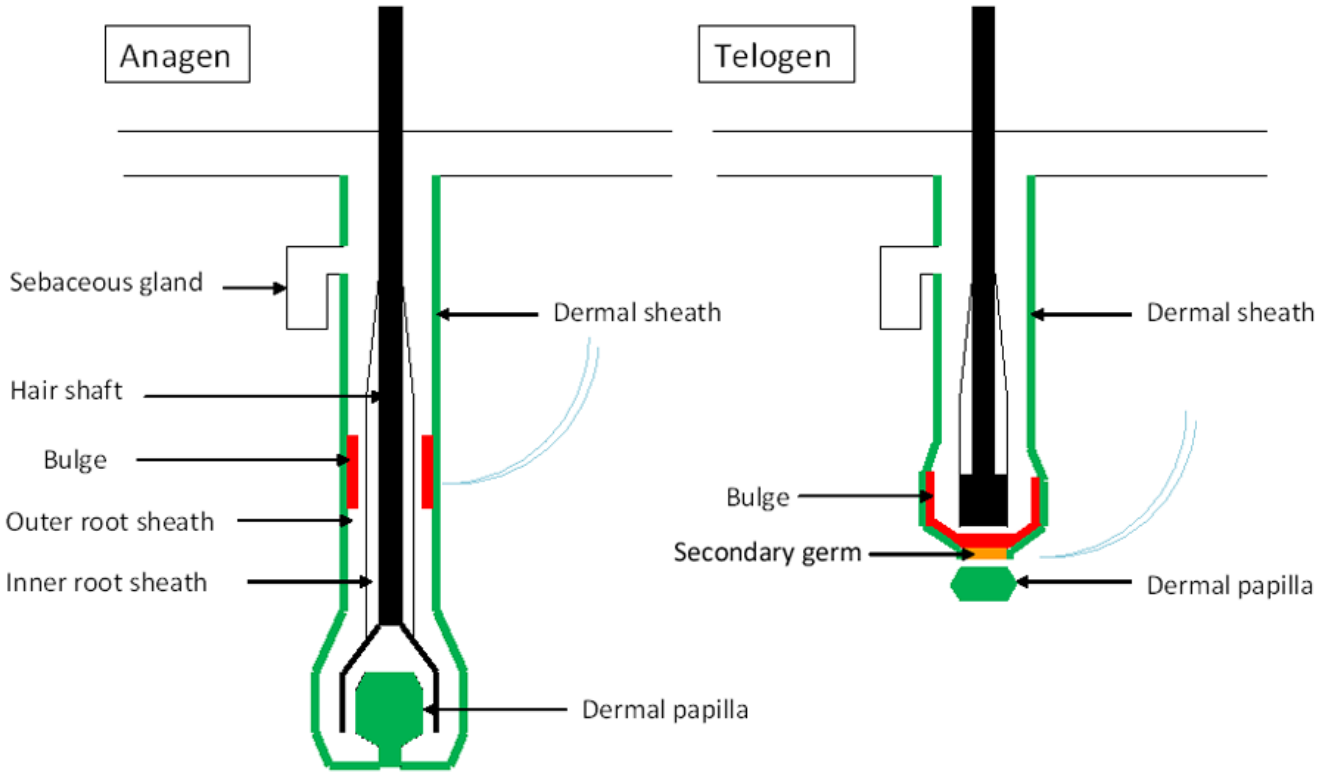


Fig. 1.

The structure of a hair follicle. The hair follicle stem cells (red) are located in a niche created by the dermal sheath and dermal papilla cells (green). In anagen, the dermal papilla leads the down-growth from the secondary germ and bulge cells to the formation of the bulb. In telogen, the dermal papilla resides adjacent to the secondary germ (yellow), which is derived from bulge stem cells during late catagen phase.

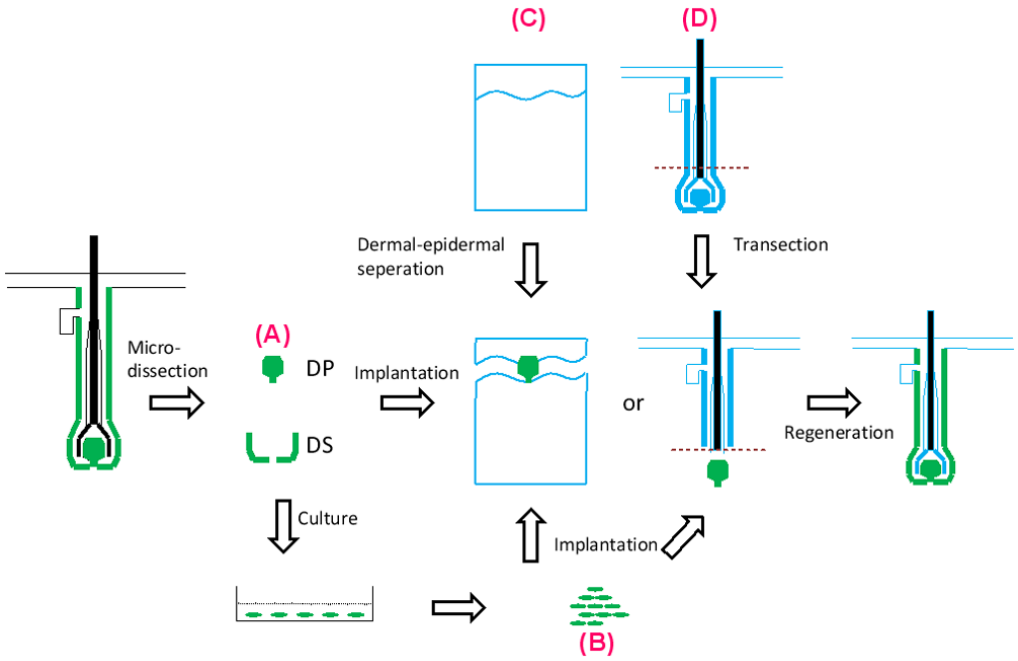


Fig. 2.

Transposition of intact dermal tissue (A) or cultured dermal cells (B) into a dermal-epidermal junctional pocket created by dispase (C) or under amputated hair follicles (D) as a model for hair regeneration.

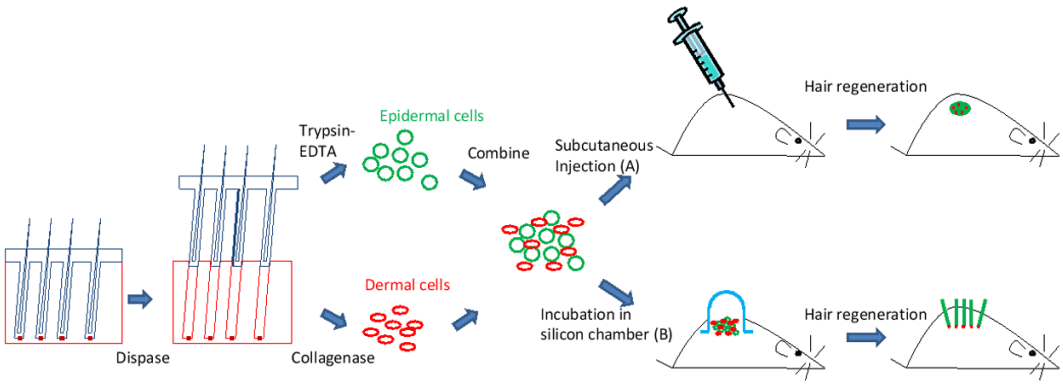
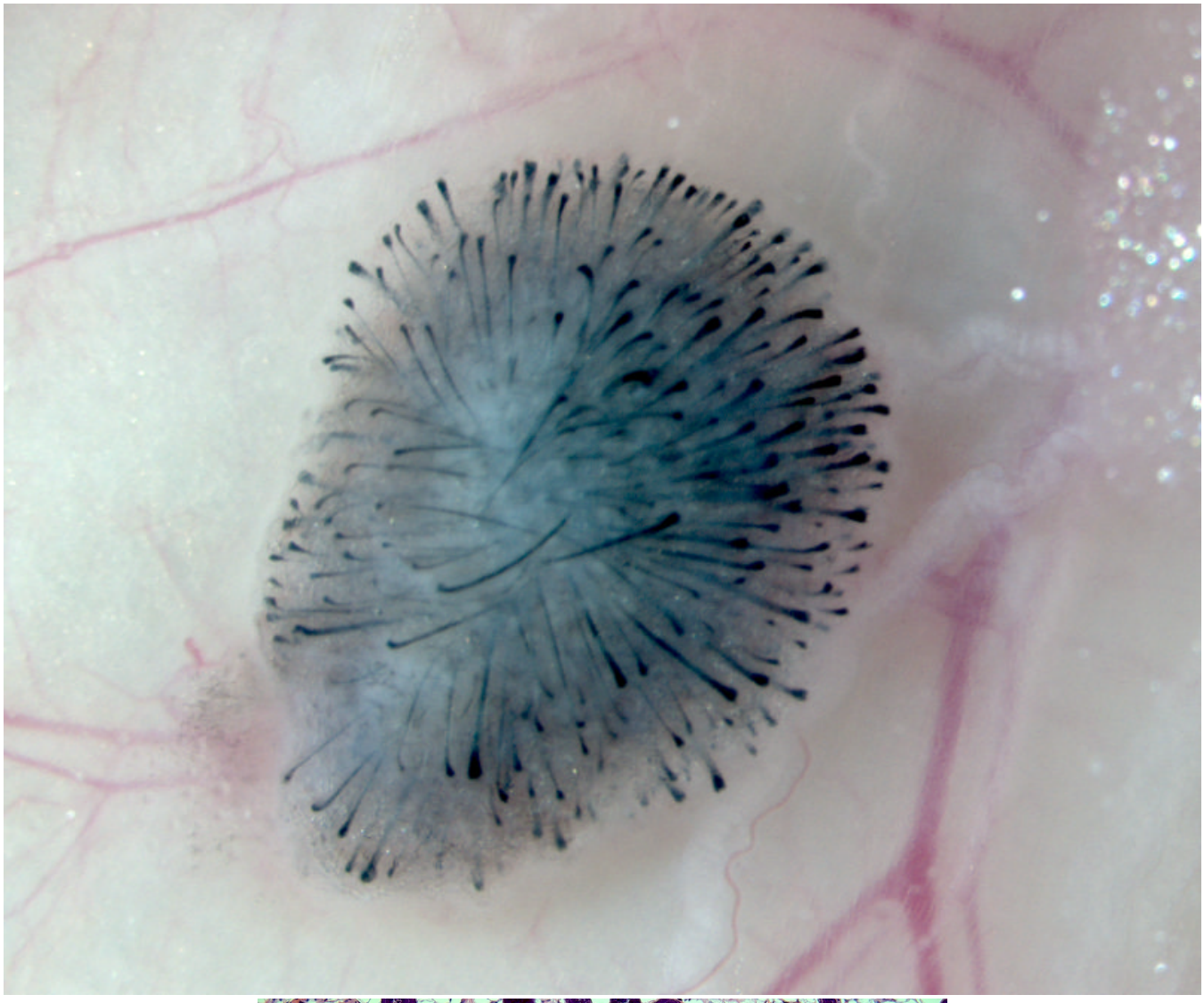


Fig. 3.

Hair regeneration models using dissociated epidermal and dermal cells. The cells are either injected subcutaneously or seeded on a full-thickness wound protected by a chamber on the back of the immunodeficient host mouse.



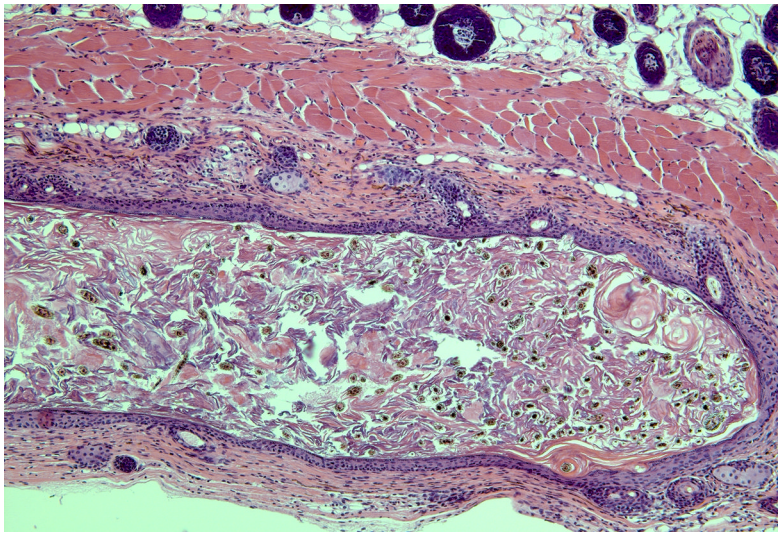


Fig. 4.

Subcutaneous injection of dissociated murine epidermal and dermal cells into the back skin of a nude mouse. (a) A nodule or “patch” with regenerated hair follicles can be observed from the undersurface of the skin in 2 weeks. (b) The histology shows formation of a cyst with hair follicles coming out from the cyst wall.

Table 1

Summary of tissue and cellular recombination assays

Author Year	Epidermal component	Dermal component	Brief methods	Results and significance
Oliver [14] 1966	Rat, transected vibrissa follicles, upper half	Rat, vibrissa DP	Implantation of DP underneath the transected follicles	Regrowth of whisker follicles after DP implantation, not without DP implantation
Oliver [43] 1970	Rat, 1–3 mm epidermal sheets from ear and scrotal skin, and lip mucosa	Rat, vibrissa DP	Implantation into rat ear skin	Vibrissa DP induce hair follicle neogenesis from epidermal sheet, even afollicular epidermis
Jahoda et al. [44] 1984	Rat, transected vibrissa follicles, upper half	Rat, cultured vibrissa DP cells (passage 1–3)	Implantation of DP underneath the transected follicles	Hair follicle neogenesis induced by cultured DP cells
Horne et al. [59] 1986	Rat, transected vibrissa follicles, upper half	Rat, **1** cultured fibroblasts (P1-3) **2** cultured DP cells (P1-15)	Implanted dermal cells beneath the transected HF	**1** The aggregative behavior leading to DP formation is only observed in early passaged DP cells **2** Interfollicular fibroblasts do not possess hair inductivity
Horne and Jahoda [11] 1992	Rat, transected vibrissa follicles, upper half	Rat, lower DS from the same follicle	Implantation of DP underneath the transected follicles	Lower DS is able to regenerate a new DP
Reynolds and Jahoda [50] 1992	Rat, adult, foot pad epidermis	Rat, cultured vibrissa DP cells	Implantation of DP cells under foot pad epidermis and transplantation into a chamber on rat skin	Hair induction in glabrous epidermis by cultured DP cells
Jahoda [51] 1992	Rat, adult, ear epidermis	Rat, vibrissa DP	Implantation of DP into small incisional wound on rat ear	Growth of vibrissa-type hair follicles supports DP's role in determining hair follicle type
Jahoda et al. [70] 1993	Rat, adult, ear epidermis	Rat, **1** cultured vibrissa DP cells **2** cultured DS cells **3** cultured fibroblasts	Implantation of cultured dermal cells into a wound create on rat ear	**1** DP cells (passage 1–3) produce abnormally large hairs **2** DP cells (passage 4–6), DS cells, and fibroblast: no hair growth
Weinberg et al. [71] 1993	Mouse, neonatal, epidermal cell aggregates (hair buds)	Rat and mouse, dissociated cells from the whole dermis	Chamber method	Regeneration of hair follicles from dissociated cells
Lichti et al. [72] 1993	Mouse, neonatal, hair buds	**1** Rat and mouse, fresh dermal cells **2** immortalized DP cells	Chamber method	**1** Immortalized DP cells induce less hair than fresh dermal cells **2** Optimal reconstitution of hair growth possibly requires the contribution from other cell types.

Author Year	Epidermal component	Dermal component	Brief methods	Results and significance
Jahoda et al. [73] 1996	Human, transected hair follicles, upper half		Subcutaneous implantation into nude mice	DS is able to replace DP
Reynolds et al. [12] 1999	Human, forearm epidermis	Human, DS from scalp	Implantation of DS into a wound on forearm	**1** Hair inductivity in human adult DS is shown **2** Trans-gender hair induction
Kishimoto et al. [62] 2000	Mouse, neonatal, dissociated cells	Mouse, neonatal, cultured dermal cells, treated with Shh, Wnt3a	Chamber method	**1** Number of induced hair follicle: Wnt3a≫Shh>control **2** Utilization of *versican*-GFP mouse to enrich inductive dermal cells
Jahoda et al. [45] 2001	Rat, transected vibrissa follicle, upper part	Human, intact DP from scalp	Recombination and implantation into nude mice	Trans-species induction of hair by human DP
McElwee et al. [13] 2003	Mouse, ear epidermis	Mouse, cultured DP, DSC, DS cells	Injection of the cellular mixture into mouse ear	Hair induction by DP and DSC cells
Morris et al. [54] 2004	Mouse, adult K15-positive cells (*K15*-EGFP)	Mouse, neonatal, dissociated dermal cells	Subcutaneous injection	Hair follicle can be generated from multipotent adult stem cells but not from the whole epidermal population
Halvickova et al. [56] 2004	Human, cultured outer root sheath cells (ORSK)	Human, cultured DP cells	Fibroblasts-incorporated collagen matrix with ORSK and DP cells in Matrigel on the top	An epithelial-mesenchy mal interaction assay system yields hair follicle-like structures.
Reynolds and Jahoda [74] 2004	Rat, transected vibrissa follicle, upper half	**1** Rat dental cells **2** Human dental cells	Implantation into kidney capsule of nude mice	Cross-appendage (hair/teeth) and interspecies (mouse/human) interaction
Zheng et al. [55] 2005	Mouse, neonatal, epidermal aggregates	Mouse, neonatal, dissociated cells	Subcutaneous injection	Mechanism of cyst formation with hair follicle neogenesis
Ouji et al. [63] 2006	Mouse, neonatal, cultured cells	Mouse, neonatal, freshly prepared cells	Chamber method, graft with Wnt-secreting cells	The importance of Wnt-10b in hair development.
Inamatsu et al. [48] 2006	Rat, small pieces of sole epidermis	Rat, **1** embryonic dermis **2** DP from vibrissa **3** cultured DP aggregates	Epidermal and dermal portions combined and grafted onto nude mouse	**1** Embryonic dermal condensates and adult DP induce hair follicles differently **2** Glabrous skin is able to form hair follicles
Osada et al. [30] 2007	Mouse, neonatal, dissociated cells	Mouse, DP cell culture	Implantation into nude mouse skin	DP cells grown in spheres induce more hair follicles
Nakao et al. [58] 2007	Mouse, embryonic dissociated epidermal cells from vibrissa	Mouse, neonatal, dissociated dermal cells from vibrissa	Organ culture in collagen gel for 2 days then transplant into	Regeneration of structurally corrected vibrissa

Author Year	Epidermal component	Dermal component	Brief methods	Results and significance
			subrenal capsule of mice	
Ehama et al. [53] 2007	Human, neonatal and adult, foreskin epidermal cells	Mouse, dissociated dermal cells (frozen and thawed)	Chamber method	Chimeric hair follicle-like structure
Ito et al. [41] 2007	Mouse, embryonic, dissociated epidermal cells	Mouse, CD133+, CD133− and whole dermal cells	Subcutaneous injection	CD133 as a marker for the isolation of hair-inducing cells
Qiao et al. [75] 2008	Mouse, embryonic, epidermal sheets	Cultured mouse embryonic dermal cells and human neonatal foreskin dermal cells	Grafted epidermal sheet plus dermal cells into nude mice under a protective skin flap	A graft model that can be used to test the hair-inductivity of dermal cells
Qiao et al. [57] 2008	Mouse, embryonic, mixture of single follicular epidermal cells and dermal cells		Suspension culture to form aggregates followed by transfer to 96-well plate to form proto-hair and transplantation to nude mice ear	In vitro epidermal-dermal aggregation and proto-hair formation with in vivo hair formation
Osada et al. [49] 2009	Mouse, foot pad epidermis	Mouse, DP cells, clones from single DP cell	Insertion of DP sphere into epidermal-derma l separation and implantation into nude mice	DP cells have intrinsic hair follicle-inducing ability