

Chapter 6

Functional Hair Follicle Regeneration

Koh-ei Toyoshima and Takashi Tsuji

Abstract The hair organ plays biologically important roles in thermoregulation, physical insulation, waterproofing, tactile sensation, protection, camouflage, and social communication. Only the hair follicle, which is induced by reciprocal epithelial and mesenchymal interactions in the skin field, has various organ-inductive potential stem cells and their niches and undergoes repeated organogenesis after birth through interactions between epithelial stem cells in the bulge region and dermal papillae. Hair loss disorders such as androgenetic alopecia are psychologically distressing and have negative effects on the quality of life. Therefore, the development of hair follicle regeneration therapy is expected to be a next-generation organ replacement regenerative therapy. Previously, many studies have reported technological approaches to reproduce de novo folliculogenesis. Recently, we successfully developed fully functional hair regeneration via intracutaneous transplantation of a bioengineered hair follicle germ, which was reconstituted from adult hair follicle-derived stem cells using our developed organ germ method. Here, we review hair follicle regeneration studies and discuss the potential of our functional hair follicle regeneration for the realization of future hair follicle organ regenerative therapy.

Keywords Hair follicle • Organ replacement regenerative therapy • Bioengineered hair follicle • Organ germ method

K.-e. Toyoshima

Department Regenerative Medicine, Plastic and Reconstructive Surgery,
Kitasato University School of Medicine, Sagamihara, Kanagawa 252-0374, Japan

RIKEN Center for Developmental Biology, Kobe, Hyogo 650-0047, Japan

Organ Technologies Inc., Tokyo 101-0048, Japan

e-mail: k-toyoshima@cdb.riken.jp

T. Tsuji (✉)

Laboratory for Organ Regeneration, RIKEN Center for Developmental Biology,
2-2-3, Minatojima-mimamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan

e-mail: t-tsuji@cdb.riken.jp

6.1 Introduction

The hair organ plays biologically important roles in thermoregulation, physical insulation from UV radiation, waterproofing, tactile sensation, protection against noxious stimuli, camouflage, and social communication (Hardy 1992; Schneider et al. 2009). The initiation of hair follicle organogenesis is induced in the hair germ through reciprocal epithelial-mesenchymal interactions and, ultimately, development into the hair follicle (Hardy 1992; Stenn and Paus 2001) (Fig. 6.1a). The hair follicle is composed of an upper permanent region and a lower variable region that

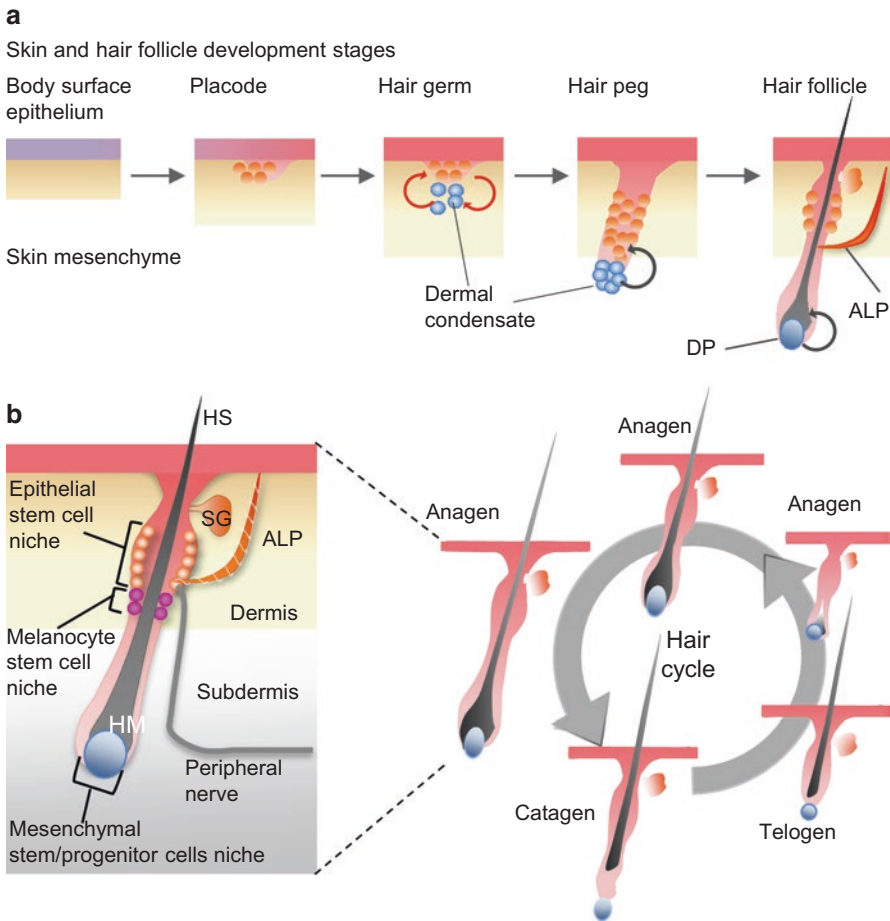


Fig. 6.1 Organogenesis of the hair follicle during embryogenesis and the hair cycle of the adult hair follicle. **(a)** Hair follicle development during embryonic skin development. *Red round arrows* and *black round arrows* represent epithelial-mesenchymal interactions and hair signaling, respectively. **(b)** Various stem cells and their niches in the adult hair follicle and hair cycle. *DP* dermal papilla, *HM* hair matrix, *SG* sebaceous gland, *ALP* arrector pili muscle, *HS* hair shaft

includes the hair bulb, which is in fact a hair shaft factory (Schneider et al. 2009; Stenn and Paus 2001). After morphogenesis, various stem cell types are maintained in particular regions: follicle epithelial cells in the follicle stem cell niche of the bulge region (Oshima et al. 2001; Hsu et al. 2014), multipotent mesenchymal precursors among DP and the dermal sheath cup (Jahoda and Reynolds 2001; Jahoda et al. 2003; Rahmani et al. 2015), neural crest-derived melanocyte progenitors in the bulge and sub-bulge region (Nishimura et al. 2002, 2005), and follicle epithelial stem cells in the bulge region connected to the arrector pili muscle (Hardy 1992; Schneider et al. 2009; Fujiwara et al. 2011) (Fig. 6.1b). These follicle stem cells contribute to repetitive regeneration of the variable region, in which hair follicle morphogenesis and the hair growth phase in postnatal hair follicles have many similar features, and both processes are characterized by the activation of cell differentiation programs that lead to the construction of hair shaft-producing epithelial hair bulbs (Schneider et al. 2009; Stenn and Paus 2001).

Hair loss disorders, such as alopecia areata and androgenetic alopecia, are psychologically distressing and have negative effects on the quality of life in both sexes (Mounsey and Reed 2009). Current pharmacological treatments do not achieve ideal control of hair loss, even in common conditions such as androgenetic alopecia or alopecia areata (Mounsey and Reed 2009). Early studies have shown that tissues or cultured cells derived from rodent dermal papilla could induce a new growth phase leading to a functionally and structurally proper hair bulb when experimentally implanted into the permanent region of the hair follicle or a follicular ear skin (Jahoda et al. 1984). Furthermore, Reynolds et al. (1999) raise the possibility that *de novo* hair follicle induction can be achieved through allogenic transplantation of hair follicle-inducible mesenchymal tissues into the skin. It has been hoped that the development of bioengineering technologies will enable future regenerative therapy for hair loss (Chuong et al. 2007).

To achieve the realization of hair follicle regenerative therapy for hair loss, it is most important to develop a highly efficient hair follicle germ regeneration technique that can provide a structurally proper and fully functional hair follicle and apply this technique for clinical usage (Chuong et al. 2007; Toyoshima et al. 2012). Over 30 years, many studies have described technologies to reconstitute the variable lower region of the hair follicle (Toyoshima et al. 2012), to reproduce *de novo* folliculogenesis via replacement with hair follicle-inductive dermal cells (Stenn et al. 2007) (Fig. 6.2a), and to direct the self-assembly of skin-derived epithelial and mesenchymal cells (Weinberg et al. 1993; Zheng et al. 2005; Stenn et al. 2007; Lichti et al. 2008) (Fig. 6.2b). These technologies provide the basic potential to reconstruct a regenerated hair follicle for hair regeneration *in vivo* (Chuong et al. 2007). However, several technical issues, including precise cell processing methods in a three-dimensional stem cell culture, eruption of a bioengineered hair by intracutaneous transplantation *in vivo*, restoration of the correct connection to surrounding tissues such as arrector pili muscle and nerve fibers, and an enduring hair cycle over a lifetime, must be resolved (Chuong et al. 2007; Lee and Chuong 2009; Toyoshima et al. 2012). Recently, a novel bioengineering method, designated the organ germ method, was developed to generate a bioengineered organ germ by multicellular

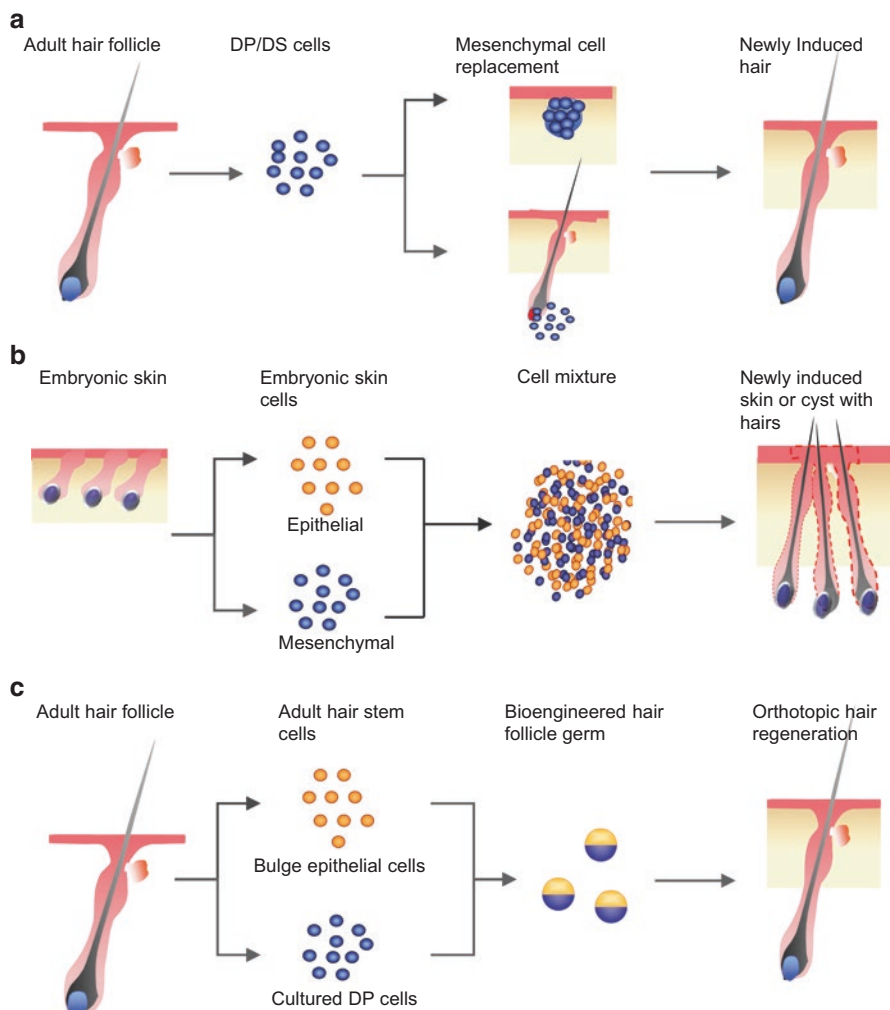


Fig. 6.2 The method used to reproduce the bioengineered hair follicle germ relied on reproducing the epithelial-mesenchymal interactions of the natural hair follicle. **(a)** Replacement of hair follicle-inductive mesenchymal cell in afollicular skin or hair follicle via the implantation of hair follicle-derived mesenchymal cells. **(b)** De novo skin regeneration method via the engraftment of cell aggregates. **(c)** Orthotopic hair follicle regeneration by intracutaneous transplantation of the bioengineered hair follicle germ reconstituted using the organ germ method

organization of epithelial and mesenchymal cells in a 3D stem cell culture (Nakao et al. 2007; Ikeda et al. 2009) (Fig. 6.2c). The bioengineered organ germs reconstituted using the organ germ method could regenerate the fully functional bioengineered hair follicles in vivo. Hair regeneration has been successfully demonstrated by intracutaneous transplantation of a bioengineered pelage and vibrissa hair follicle germ or ectopically regenerated mature hair follicle, which was regenerated with

embryonic skin-derived cells and adult vibrissa follicle-derived stem cells, respectively (Toyoshima et al. 2012; Asakawa et al. 2012).

In this chapter, we describe the features of the hair follicle as a main target of organ regenerative therapy for the medical cure of alopecia and the changing in technical developments. We also provide several results using bioengineered hair follicle germs for hair regeneration and a perspective for the realization of organ regenerative therapy of the hair follicle.

6.2 Organogenesis of the Hair Follicle During Embryogenesis

Hair follicle organogenesis, in principle, takes place in the developing skin (Hardy 1992; Stenn and Paus 2001; Fuchs 2007; Schneider et al. 2009) (Fig. 6.1). When the embryonic epidermis and dermis are both intact, complex signaling between them is initiated that leads to fate changes in both tissue layers, ultimately resulting in epidermal patterning and the development of a hair follicle (Paus 2007; Benitah and Frye 2012). To better understand this complex biological multicellular system and the technical and clinical subjects, both hair follicle organogenesis and regeneration processes should be reconsidered in light of the notion of self-organization, which is found universally in nature and defined as the spontaneous formation of ordered patterns and structures from a population of elements with no or minimal patterns (Sasai 2013a). Sasai has advocated that the principle process of biological self-organization can be defined and classified into the self-assembly stage, self-patterning stage, and self-driven morphogenesis (Sasai 2013a).

In the context of the notion of biological self-organization, primitive skin develops as a hair follicle organogenetic field at the preceding outset of hair follicle organogenesis (Fuchs 2007; Lim and Nusse 2013). Post-gastrulation, the embryonic surface cells emerge as an ectodermal cell monolayer that is regionally specified and differentiates into the neurogenic and body surface ectoderm, which will ultimately develop into the central nervous and integumentary system (i.e., skin organ system) (Fuchs 2007; Lim and Nusse 2013). Mesoderm-derived mesenchymal cells underlie the body surface ectoderm (Fuchs 2007). Hair morphogenesis initiates the specialization of the epidermis at regularly spaced intervals, leading to the formation of epidermal placodes (Lim and Nusse 2013) (Fig. 6.1a). A large number of studies examining hair follicle patterning have focused on a reaction-diffusion model to understand the underlying mechanisms. Canonical Wnt/ β -catenin signaling in the ectodermal epithelium and its inhibitors behave as a reaction-diffusion model resulting in an activation status or a lack of β -catenin expression, thus determining the primary hair follicle spacing and distribution (Lim and Nusse 2013). Subsequently, almost immediately after placode formation, the mesenchymal cells underlying the placode give rise to a cluster and condense into a dermal condensate (Hardy 1992; Fuchs 2007; Schneider et al. 2009). The signals from the dermal

condensates induce epidermal placode cells to rapidly divide in a downward direction and invade the dermis, enwrapping the dermal condensate, which becomes the dermal papilla (Carlsen 1974; Hardy 1992; Schneider et al. 2009; Wang et al. 2012).

Wnt signaling is essential for inducing the dermal condensate (Atit et al. 2006; Ohtola et al. 2008; Lim and Nusse 2013). The expression profile of the Wnt family in the homogeneous primitive ectodermal epithelium has been evaluated by *in situ* hybridization, indicating that Wnt gene expression patterns in the embryonic skin epithelium during the placode promotion stage are classified as uniformly expressing Wnt3, 4, and 10a, localizing Wnt10b in the placode (Andl et al. 2002). Wnt10b signaling regulates the differentiation from neural crest-derived mesenchyme into DP and the surrounding fat tissue (Fu and Hsu 2013; Ouji et al. 2013; Ross et al. 2000). Wnt10b is a critical epithelial signal for fate decisions in the hair follicle mesenchymal cell population (Lim and Nusse 2013). The dermal condensate induces the placodal epithelium to proliferate, develop into a multilayer, and invade the dermis, resulting in the formation of a hair germ (Hardy 1992; Stenn and Paus 2001; Schneider et al. 2009; Wang et al. 2012).

The canonical process of hair follicle development after the hair germ stage is thought to be a self-driven morphogenesis process because isolated embryonic skin fragments, including the hair follicle germ, can progressively develop into a hair follicle in an *in vitro* organ culture without intact hormonal and neural circuits (Hardy 1949; Schneider et al. 2009). Elongation of the hair germ and augmentation of cell proliferation at the proximal end of the hair peg toward the surrounding dermal condensate, which will become dermal papilla, are evoked by the reciprocal dermal signaling from the hair peg to the bulbous hair peg stage (Hardy 1992; Stenn and Paus 2001; Schneider et al. 2009). In the latest stages of folliculogenesis, the structurally mature hair follicle has two distinct components, a variable region, which comprises the portion growing downward that contains the hair bulb referred to as the actual hair factory, and a constant region (Schneider et al. 2009). The constant region of the hair follicle connects to the peripheral nerve and arrector pili muscle and functionally matures in the postnatal skin (Paus et al. 1999) (Fig. 6.1b). Human hair follicle development is first recognizable in the fetal craniofacial region of the skin by 9–10 weeks gestational age (GA), and it globally progresses throughout organogenesis of hair follicle by 20 weeks GA (Carlsen 1974; Akiyama et al. 2000). Interestingly, similar to rodent vibrissae, the upper lip portion of the human fetus precedes that of any other region of the body in terms of hair follicle organization (Akiyama et al. 2000).

6.3 Hair Cycle of the Adult Hair Follicle

After morphogenesis in the postnatal stage, the hair follicle undergoes the hair cycle of the regression (catagen), resting (telogen), and reproduction of hair follicle organogenesis and hair growth (anagen) phases throughout the lifetime of the organism (Fig. 6.1b) (1–3). A cyclical recapitulation of organ regeneration similar to that

of the hair follicle is observed only in the case of deer antlers (Randall et al. 1993; Thornton et al. 1996; Polykandriotis et al. 2010). The germinative epithelial cells in the proximal region of the hair matrix are described as transit-amplifying cells because they only survive through anagen (Reynolds and Jahoda 1991 and 1996). At the end of anagen, cell division in the germinative hair matrix supplies the hair differentiation of the hair shaft and the slowly developing IRS, and consequently the hair follicle enters catagen (Stenn and Paus 2001; Millar 2002). During catagen, the entire variable region is reduced to an epithelial column to form a secondary hair germ, mainly by apoptosis of matrix, IRS and ORS epithelial cells, while bulge hair follicle stem cells evade apoptosis (Millar 2002). Following catagen, the hair follicles enter a telogen phase, which is acknowledged as a relatively quiescent and resting phase (Stenn and Paus 2001; Millar 2002; Schneider et al. 2009).

The initiation of a new anagen phase in the adult hair cycle and embryonic folliculogenesis are appreciated for sharing many features that are considered, at least in part, to be different phenomena (Stenn and Paus 2001; Botchkarevn and Kishimoto 2003; Schneider et al. 2009). The processes are distinguished as follows: one is the organogenesis of a whole hair organ, and the other is the partial reproduction of a variable region of adult hair follicle (Botchkarevn and Kishimoto 2003; Stenn et al. 2007). Both processes are characterized by the induction and control of cell proliferation and differentiation to construct the hair-producing system and maintain hair growth through epithelial-mesenchymal interactions, which are mediated by similar signaling molecules (Botchkarevn and Kishimoto 2003). During early anagen, the secondary hair germ forms a hair bulb, which grows progressively downward and away from the bulge region (Botchkarevn and Kishimoto 2003). Although the ORS cells of the bulge region readopt a quiescent, undifferentiated epithelial cell phenotype in the lower portion of the hair matrix, the proliferative potential is maintained through active Wnt signaling from dermal papilla cells and consequent stabilization of β -catenin in matrix epithelial cells throughout anagen (Stenn and Paus 2001; Botchkarevn and Kishimoto 2003; Schneider et al. 2009). In the upper part of the hair matrix, proliferation is arrested and terminal differentiation is initiated (Botchkarevn and Kishimoto 2003). Many pioneering investigations have revealed multiple molecules that regulate hair lineages, such as Wnt/b-catenin/Lef1, Notch1/Jagged/Delta, BMPs/BMPRIa, Msx and Foxn1 for hair shaft development and IGF1 and HGF production, and VEGF for the maintenance of anagen (Botchkarevn and Kishimoto 2003; Schneider et al. 2009; Lim and Nusse 2013).

The anagen-catagen transition, which is associated with a remarkable decrease in the exchange of epithelial-mesenchymal interaction signals in anagen, determines the hair length of the corresponding hair types (Randall et al. 1993; Hibberts et al. 1998). Several studies suggest that this transition timing is controlled by an internal biological clock because ectopically transplanted hair follicles can retain their own inherent hair cycling (39). During catagen, the lower part of the hair follicle rapidly regresses via apoptosis of epithelial cells as a consequence of the increased expression of apoptosis stimulation factors, such as FGF5, TGF β , TNF α , and neurotrophin (Stenn and Paus 2001; Botchkarevn and Kishimoto 2003; Wang et al. 2012). Upon completion of catagen, the hair follicle enters telogen, which can be separated into

two stages: refractory to the induction of anagen and a competent stage wherein secondary hair germ cells become highly sensitive to anagen-inducing factors (Plikus et al. 2008). The BMP2 and 4 signals derived from dermal papilla cells inhibit activation of the Wnt/ β -catenin pathway in the epithelial cells of the secondary hair germ (Plikus et al. 2008). Conclusive studies have demonstrated that extra-follicular BMP signals, which are provided by undifferentiated progenitors of adipocytes in the subcutaneous adipose, also override the entry of competent telogen into anagen and are negatively regulated by epithelial adipogenetic factors (Plikus et al. 2008; Festa et al. 2011; Rivera-Gonzalez et al. 2014; Hsu et al. 2014). Additionally, several observations have suggested that the skin peripheral nerve connected to the hair follicle controls enlargement of the hair follicle during anagen (Peters et al. 2006; Hsu et al. 2014).

6.4 Epithelial and Mesenchymal Stem Cells in the Adult Hair Follicle

To achieve iteration of the hair cycle, maintenance of the various adult stem cells and their niches in the mature hair follicle is considered to be essential throughout the lifetime of the organism (Cotsarelis et al. 1990; Greco et al. 2009). The most essential hair follicle stem cells (i.e., the minimal cell elements for the reconstruction of the entire hair follicle) are epithelial and mesenchymal stem cells (Botchkarev and Kishimoto 2003). When the lower half of the hair follicle is surgically ablated, the remaining upper portion cannot regenerate a hair bulb (Jahoda et al. 1984; Horne et al. 1986). Surgical destruction of the area between a sebaceous gland and the arrector pili muscle connecting the portion of the human hair follicle, which is referred to as the bulge region, causes irreparable damage to the hair follicle and is applied clinically for hair removal operations (Unger et al. 2010). Moreover, reconstruction experiments of the bulge epithelium and dermal papilla or dermal sheath cup demonstrated that a structurally and functionally complete hair bulb can be regenerated by reproducing the epithelial-mesenchymal interactions, similarly to anagen of the natural hair follicle (Jahoda et al. 1984; Horne et al. 1986). These observations directly indicate that the hair follicle epithelial stem cells and mesenchymal stem cells are localized in stem cell niches in the bulge region and lower variable region, respectively (Lavker et al. 2003; Schneider et al. 2009).

The major biological characteristics of stem cells *in vivo* are quiescence, a high proliferative capacity, and multipotency to produce multiple lineage cells (Claudinet et al. 2005a, b). Slow cycling epithelial cells of the adult hair follicle are detected as label-retaining cells in the bulge region, and lineage analysis has shown that all follicular epithelial cells originate from bulge ORS epithelial cells (Oshima et al. 2001; Claudinet et al. 2005a, b; Ohyama et al. 2006; Waters et al. 2007). Several researchers have reported that certain markers, such as cytokeratin 15 (CK15), CD34, and

CD49f, are preferentially expressed in bulge ORS cells of the murine pelage hair follicle (Claudinot et al. 2005a, b; Wang et al. 2012). The combination of antibodies recognizing the cell surface molecules of bulge cells and transgenic mice that express fluorescent reporter proteins under the control of these markers provides a means for the characterization and isolation of living bulge cells (Ohyama et al. 2006). Living bulge cells that are enzymatically dissociated into single cells have enabled stem cell biologists to quantitatively evaluate in vitro colony-forming ability and to analyze the clonogenic cell dynamics of hair epithelial stem cells (Ohyama et al. 2006). Subsequent studies have indicated that hair follicle epithelial stem cells can differentiate into not only hair follicles but also the interfollicular epidermis (Oshima et al. 2001). The multiple subpopulations of epithelial stem cells and progenitor cells, which are distinguishable by their expression of specific molecules such as Sox9, LGR5, LGR6, LRIG1, and Gli1, have been shown to be structurally and functionally organized as a cellular hierarchical system in the murine pelage bulge region (Morris et al. 2004; Vidal et al. 2005; Jensen et al. 2009).

In the variable region of the hair follicle, two histologically distinct mesenchymal cell populations have been shown: the dermal papilla and the dermal sheath (Hardy 1992; Jahoda and Reynolds 2001; Rahmani et al. 2015). The dermal papilla is surrounded by the hair matrix epithelium and continues histologically into the dermal sheath, which consists of loose connective tissue overlying the outermost hair follicle (Hardy 1992; Rahmani et al. 2015). Isolated dermal papilla cells can be propagated in an in vitro culture system (Jahoda et al. 1984; Osada et al. 2007). Previous studies have indicated that freshly isolated and cultured adult dermal papilla cells provide unique and critical signals for the induction of anagen (Stenn and Paus 2001; Botchkarevn and Kishimoto 2003). Mesenchymal stem/progenitor cell populations, which can differentiate into dermal sheath, adipose, cartilage, and dermal fibroblasts, are found among the dermal papilla (Jahoda et al. 2003). However, analysis of the cell dynamics of the dermal papilla and dermal sheath cells suggested that dermal sheath cells proliferate and migrate to provide the dermal papilla cells (Rahmani et al. 2015). In contrast to follicular epithelial stem cells, hair mesenchymal cells have been shown to express versican, alkaline phosphatase (ALP), and α -smooth muscle actin (α SMA) as relative specific markers for the dermal papilla and dermal sheath cells (Kishimoto et al. 1999). However, an in vivo fate mapping study of adult hair follicle dermal sheath cells using α SMA-fluorescent transgenic mice indicated that dermal sheath cup cells possess critical stem cell properties (Rahmani et al. 2015). ALP activity is strongly detected in the dermal papilla in early anagen, although differences in ALP activity between these components increase during all other hair cycle phases (Iida et al. 2007). It has been suggested that the hair follicle-inducing ability of dermal papilla cells is closely related to ALP expression (Iida et al. 2007). These findings indicate that the cellular heterogeneity of dermal papilla cells, which consists of various stages of cell commitment, may be altered and governed according to the hair cycle phases (Iida et al. 2007).

6.5 The Human Hair Follicle as a Clinical Target of Organ Regenerative Therapy

Human hairs are commonly classified into a terminal hair and a vellus hair, according to the diameter, length, and internal structure (Hojiro 1972; Driskell et al. 2009). The temporal parameters of the hair cycle are dependent on the types of hairs and on the means of the maximum length and density of the hair shaft (Toyoshima et al. 2012). On the typical normal human scalp, there are 100,000 terminal hairs, approximately 90% of which are normally in anagen and 10% in telogen (Ramos and Miot 2015). During the late fetal stage to birth, fetal hair follicles are converted from a lanugo type to mainly terminal hair and vellus hair types, or they involute into vestigial organs in the labial gingiva (Akiyama et al. 2000). In the context of hair type conversion, terminal hairs grow at secondary sexual sites before puberty (Randall et al. 1993). It is thought that a single hair follicle retains the inherent hair type and the biological program of hair type conversion determined by fate determination during hair follicle organogenesis, although the underlying mechanism remains unexplained (Randall 1992; Thornton et al. 1996).

Throughout the human lifetime, although up to 50% of the population is affected by hair loss, which is a common symptom of various hair-related disorders, traumatic injury, psychiatric disorder, and age-related physiological changes, 95% of clinical hair loss in men and women is caused by androgenetic alopecia (Mounsey and Reed 2009). It is possible that hair loss can occur anywhere in human skin, although most patients commonly complain of terminal hair trouble, especially scalp hair loss (Mounsey and Reed 2009). Based on the focal pathosis of alopecia, it is clinically valuable to divide hair loss into an acute or a chronic scarring alopecia and a non-scarring alopecia (Mounsey and Reed 2009; Qi 2015; Knopp 2015). Acute scarring alopecia is characterized by extrinsic causes, such as active inflammation, irreversible destruction of the hair follicle, and fibrous tissue replacement of hair follicles, and it requires a swift effective medical cure during early disease stages. In contrast, non-scarring alopecia is characterized by significant alterations in follicular size, abnormalities in the hair cycle, and reversible anatomical changes in the follicles (Sperling 2001; Mounsey and Reed 2009). The stable region of the hair follicle is commonly irreversibly degenerated or damaged in scarring alopecia (Sperling 2001).

Androgenetic alopecia in humans is pathologically characterized by a gradual reduction in size and shortening of the growth phase of focal hair follicles, which are intrinsically fate determined and genetically programmed to alter vellus hair types mediated by the increasing expression of 5 α -reductase and the conversion of testosterone into dihydrotestosterone in the dermal papilla cells of susceptible hair follicles (Randall et al. 1993). Thus, the surgical approach to androgenetic alopecia, which is based on follicular unit transplantation (FUT) and these minimal surrounding tissues dissected from the normal scalp to focal areas, can provide highly clinically effective outcomes for male and female patients (Unger et al. 2010). Drugs targeting 5 α -reductase, which can inhibit a trigger of the intrinsic program of

androgenetic alopecia, are also useful for the clinical treatment of male pattern baldness, but they have marginal effects in female patients (Mounsey and Reed 2009; Unger et al. 2010; Qi 2015; Knopp 2015). These observations suggest that androgenetic alopecia arises from alterations of the dermal papilla cells and may reflect a primary aberration in either the hair follicle compartment or its surrounding tissues (Rivera-Gonzalez 2016).

Several lines of evidence from animal experiments indicate that the hair loss pattern is attributed to the differential embryological origins of the frontal and occipital scalp mesenchyme (Qi and Garza 2014). The neural crest-derived mesenchymal cells exhibit extensive diversity in molecular markers that depend on the embryonic development stages (Qi and Garza 2014). Recently, neural crest-derived dermal stem cells, which have been defined as multipotent stem cells that are enriched in the dermal sheath, can differentiate into the entire hair follicular mesenchyme and the subcutaneous adipose (Qi and Garza 2014). The undifferentiated adipogenic progenitors in the subcutaneous tissue turn off the termination of hair follicle telogen phase, resulting in expansion of the telogen phase (Qi and Garza 2014). Conversely, during the transition from telogen to anagen, hair follicle epithelial cells induce progenitor differentiation into adipocytes, which can trigger the initiation of anagen (Qi and Garza 2014). These findings suggest that a capability of the interaction between the hair follicle and adipose tissue is a modulation of the duration of telogen, a synchronous enlargement of mature adipose tissue with hair cycling, and a differential reinforcement of dermal fibrosis, corresponding to the pathology of chronic scarring alopecia (Qi and Garza 2014).

6.6 Methodology for the Reconstruction of a Fully Functional Bioengineered Hair Follicle

The reproduction method for the bioengineered hair follicle germ relies on the ability to reproduce the epithelial-mesenchymal interactions that occur during embryonic organogenesis, and adult cyclic regeneration of the hair follicle is essential for fundamental hair follicle organ regeneration strategies (Chuong et al. 2007). To establish *de novo* regeneration of functional hair follicle, several animal models of hair formation are available and can be applied to dissect intact or partial hair follicle tissues, to culture follicular cells and tissues, and then to orthotopically or ectopically transplant them into an immunodeficient or syngeneic mouse (Chuong et al. 2007; Toyoshima et al. 2012). The orthotopic transplantation model can be used to visually estimate hair growth and continuous hair cycling of the bioengineered hair follicle in living animals (Chuong et al. 2007; Toyoshima et al. 2012). Oliver and colleagues demonstrated that a secondary hair follicle germ could be artificially reconstructed with the constant region of the host hair follicle and isolated dermal papilla cells and newly induce hair bulb and hair shaft growth (Oliver 1966; Jahoda et al. 1984; Horne et al. 1986) (Fig. 6.2a). The replacement of

hair-inductive mesenchymal cells in an amputated hair follicle has served as an analogy for the reproduction of adult anagen (Jahoda et al. 1984). Many researchers have reported that the replacement of dermal cells in a follicular skin using either fresh or in vitro-expanded follicular mesenchymal cells, which are collected from adult hair bulbs in an anagen hair follicle, can newly induce hair follicle formation (Oliver 1966; Jahoda et al. 1984). In the context of mesenchymal cell replacement, Reynolds and colleagues provided the most dramatic findings for human hair follicle regeneration, which was achieved by allogeneic transplantation of connective tissue sheath tissues dissected from the male scalp hair follicle into the proximal epithelium of female forearm skin (Reynolds et al. 1999) (Fig. 6.2a). Although newly induced small hair follicles, including male donor-derived dermal papilla cells and thin hair growth, were found at the implanted site, the origin and hair-forming ability of the intrafollicular epithelial cells was not clear (Reynolds et al. 1999).

Based on the three categories of biological self-organization processes as described above, the three-dimensional cellular arrangement of the hair follicle organ germ is essential for inducing a functional hair follicle and achieved using a self-assembly process or artificial manipulation of follicular epithelial cells and mesenchymal cells (Sasai 2013a). The de novo hair regeneration methods in vitro and in vivo that rely on the reproduction of epithelial-mesenchymal interactions during the embryonic folliculogenesis and growth phases of the adult hair cycle have been attempted in several previous studies using isolated epithelial and mesenchymal cells (Weinberg et al. 1993; Zheng et al. 2005; Lichti et al. 2008). It has also been reported that dissociated follicular epithelial and mesenchymal cells are aggregated to form the hair follicle germ through a self-assembly process and that this model can utilize dermal and epidermal candidate cells (Sasai 2013b) (Fig. 6.2b). The most widely used de novo hair growth model is the silicone chamber assay (Weinberg et al. 1993; Lichti et al. 2008). In this model, the slurry of the target cell mixture is injected inside grafting chambers that have been surgically implanted into an excision area on the back of the mouse. As a positive control, 10^7 epidermal and dermal cells each derived from newborn mice induce the de novo development of hairy skin in each chamber at approximately 3 weeks after grafting. This model is useful for evaluating the follicular formation ability, and either cell component can be replaced with candidate cells such as multipotent keratinocytes isolated from adult mice (Mannik et al. 2010), cultured dermal papilla cells (Lichti et al. 2008), and human keratinocytes (Ehama et al. 2007).

The silicone chamber assay is limited by the requirement for the surgical implantation of a special apparatus (Lee and Chuong 2009). Surgical difficulties and hair regeneration efficiency were improved by the development of the patch assay, in which a similar high-density mixture of $>10^5$ dissociated epithelial and mesenchymal cells was injected at certain ratios into the hypodermis of host mice (Zheng et al. 2005) (Fig. 6.2b). The multipotency of CK15-positive epidermal cells isolated from transgenic mouse skin, including pelage hair follicles, was assessed by combining them with neonatal dermal cells in the patch assay (Zheng et al. 2005; Stenn et al. 2007). Furthermore, the hair-inducing abilities of follicular cells from various mammalian species and transgenic animals and under different culture conditions

can be evaluated using this assay model (Stenn et al. 2007; Ehama et al. 2007; Mannik et al. 2010). Using the rotation-culture method, aggregated cells from the neonatal rodent skin epithelium and mesenchyme can induce immature hair follicles in vitro and after transplantation into a receptive animal and also readily regenerate mature hair follicles using the silicon chamber and patch assays (Ihara et al. 1991; Takeda et al. 1998). Limitations of the patch assay and in vitro rotary culture assay are that the de novo hairs grow into the inside of cystic structures that are not associated with the natural skin, making it difficult to observe the hair growth without surgical dissection; the control of the orientation and direction of the bioengineered hair follicles, which are generally random; and the lack of restoration of the extra-follicular environment (Lee and Chuong 2009; Toyoshima et al. 2012). Although these bioengineering techniques provide an easy practicable model for examining hair follicle formation, the phenomena cannot be directly translated clinically because the self-assembly of epithelial and mesenchymal cells into the bioengineered hair follicle germ is inefficient and requires a large number of cells and an uncontrollable hair density and arrangement to achieve the clinical application of organ regenerative therapy for the hair follicle (Toyoshima et al. 2012).

6.7 Functional Hair Follicle Regeneration

As discussed above, before attaining clinically effective hair regeneration in human skin, technological breakthroughs for the major obstacles must be achieved by unravelling practical high-throughput methods to reproduce the bioengineered hair follicle germ and developing an in vivo evaluation model that is not only widely appropriate for various functions of the bioengineered hair follicle but also readily available for clinical application (Lee and Chuong 2009). To achieve useful hair follicle regeneration for a clinical cure, the bioengineered hair organ should have a structurally correct architecture and result in a fully grown hair shaft with a histologically proper arrangement and connection in the skin (Chuong et al. 2007; Lee and Chuong 2009); have a repeated enduring hair cycle, which is considered essential for the regeneration of the various stem cells and their niches; and exhibit the cooperative functions of the hair organ in the natural skin environment (Toyoshima et al. 2012). The organ germ method has facilitated these goals, particularly the practical high-throughput assay (Chuong et al. 2007; Lee and Chuong 2009; Toyoshima et al. 2012). The bioengineered hair follicle germ is thought to spontaneously drive organ development via an intrinsic folliculogenesis process that is reproduced from hair-inductive epithelial and mesenchymal stem cells by cell manipulation techniques to generate a highly efficient and useful hair follicle regeneration model for clinical applications (Toyoshima et al. 2012). Nakao et al. have previously developed an organ germ method and demonstrated that a bioengineered hair follicle germ, which was reconstituted with dissociated epithelial and mesenchymal cells (approximately 50,000–100,000 cells each) derived from the

embryonic hair germ of murine vibrissae, can ectopically regenerate a bioengineered hair follicle producing a hair shaft under the kidney capsule (Nakao et al. 2007; Asakawa et al. 2012) (Fig. 6.2c).

6.7.1 Hair Follicle Regeneration by Intracutaneous Transplantation of the Bioengineered Hair Follicle Germ

It is essential to evaluate whether the bioengineered hair follicle germ can develop into a structurally proper bioengineered hair follicle in the adult intracutaneous environment to provide fully functional hair organ regeneration, including hair eruption growth, hair cycles, and connections with surrounding tissues, based on the functional regeneration of stem cell niches (Toyoshima et al. 2012). Toyoshima et al. successfully demonstrated that the bioengineered hair follicle germ was reconstituted with considerably small number of adult murine vibrissa hair follicle-derived epithelial stem cells (10^4 cells) and cultured dermal papilla cells (3×10^3 cells) using an organ germ method and regenerated the hair follicle in the murine skin environment through the novel development of the intracutaneous transplantation method, which was based on the FUT surgical operation procedure for ready availability for clinical application (Toyoshima et al. 2012; Tezuka et al. 2016) (Fig. 6.2c and 6.3).

Surgical specialists in FUT therapy generally dissect normal hair follicles that maintain their structural integrity in an epithelium of the hair opening and hair shaft and then translocationally engraft them into the bald region by maintaining the external direction so that it protrudes from the skin surface to achieve a high engraftment efficiency and no significant adverse events, such as serious infectious disease and postoperative cyst formation (Toyoshima et al. 2012; Tezuka et al. 2016). To prevent posttransplant cyst formation by the rapid closing between the host skin epithelium and the bioengineered hair follicle germ, as a guide for the direction of the infundibulum, an inter-epithelial tissue-connecting nylon thread is highly effective for the accurate arrangement of the newly formed hair pore of the bioengineered hair follicle to the surrounding host skin epithelium (Toyoshima et al. 2012; Tezuka et al. 2016) (Fig. 6.3). In cases of bioengineered vibrissa follicles, the bioengineered hair shafts erupt at a resulting frequency of 74% at 21 days after engraftment and are almost unpigmented (Fig. 6.3). These results raise the possibility not only of hair organ regeneration in the adult skin environment but also reestablishment of the connections between the bioengineered hair follicle and the recipient skin, similar to the results obtained using FUT.

It is preferable for a clinically applicable hair regeneration therapy to utilize autologous FUT therapy, which is the most popular and effective surgical cure for various types of hair loss. The density, area, and direction of grafting of follicular units can be practically controlled in ranges to achieve therapeutic goals (Toyoshima et al. 2012). In the preparation of bioengineered follicle germs, it has been indicated

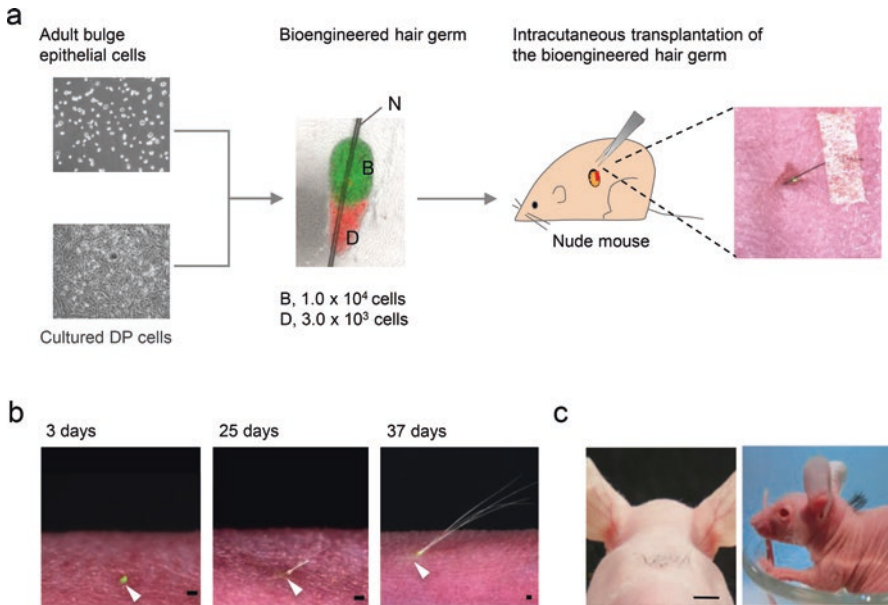


Fig. 6.3 Orthotopic hair follicle regeneration through intracutaneous transplantation of the bioengineered hair follicle germ. **(a)** Schematic representation of a preparation of the bioengineered hair follicle germ reconstructed with adult vibrissa and intracutaneous transplantation after insertion of a nylon thread as a guide for the direction of the infundibulum via insertion into the bioengineered germ. **(b)** Macro-morphological observations of the eruption and growth of bioengineered vibrissa hair shafts at day 3 after transplantation; eruption and growth of the hair shaft at days 25 and 37. **(c)** Controllable bioengineered hair density through high-density intracutaneous transplantation of the bioengineered follicle germs. A total of 28 independent bioengineered pelage hair follicle germs were transplanted into the neck skin of nude mice, and they displayed high-density hair growth at 21 days after transplantation (*left*) and a few weeks after hair eruption (*right*). Scale bar, 1 cm

that the number of bioengineered hair follicles correlates with the cell number in the bioengineered hair follicle germ and the contact area between the epithelial and mesenchymal cells. These results suggest that the number of bioengineered vibrissa follicles depends on the quantity of follicle-forming cells in bulge-derived epithelial and primary-cultured DP cells, and they show that this procedure can provide a precise transplantation technology for future hair regeneration therapy. To achieve hair follicle regeneration at hair densities of 120 hairs/cm² in normal scalp or 60–100 hairs/cm² using FUT treatment, the 28 bioengineered hair germs were successfully transplanted into an approximately 1 cm² occipital skin region and erupted 21 days after grafting at a high density of 124.0 ± 17.3 hair shaft cm² (Fig. 6.3). These results indicate that, similar to FUT therapy, the transplantation of bioengineered hair follicle germs can be applied as a treatment for androgenic alopecia (Toyoshima et al. 2012; Tezuka et al. 2016).

6.7.2 *Reproduction of the Hair Follicle Architecture and Hair Cycle*

Toyoshima et al. also analyzed the hair cycles of the bioengineered pelage and vibrissa shafts that had erupted from bioengineered follicles in immunodeficient murine skin over 80 days. The bioengineered pelage and vibrissa follicles repeated the hair cycle at least 3 times during the 80-day period, and no significant differences in the hair cycle periods were found between the natural and bioengineered follicles. These results indicated that the bioengineered hair follicle could undergo proper hair cycles according to the cell types of origin. It has been suggested that the bioengineered pelage and vibrissa follicles can reproduce these hair cycles, which are maintained by stem cells and provide a stem cell niche. The distinct hair follicle types, which are classified into awl/auchene, guard, zigzag, and vibrissa hair shafts in murine skin based on properties such as length, thickness, kinks, and hardness, are thought to be specified by dermal papilla cell types through communication between DP cells and overlying epithelial cells. The bioengineered pelage follicle germs are found to produce all types of pelage hairs, as confirmed by the hair type-specific structural properties observed by light microscopy, in accordance with the follicle fate determined during embryonic development (Toyoshima et al. 2012; Asakawa et al. 2012). The bioengineered vibrissa follicle germ regenerated a vibrissa-type hair shaft with the appropriate structural properties at the light microscopic level (Toyoshima et al. 2012). Ultrastructural observation of the bioengineered hair shafts revealed the correct reproduction of the morphological and histological structures, including the hair medulla, cortex, and cuticle.

The bioengineered hair follicle formed the correct structures, comprising an infundibulum and sebaceous gland in the proximal region as well as a hair matrix, hair shaft, IRS, ORS, and dermal papilla (Fig. 6.4). The bioengineered vibrissa follicle germs can regenerate not only the variable region but also the infundibulum and sebaceous gland in the permanent region. By contrast, the vibrissa follicle-derived cells were not distributed among the surrounding cutaneous tissues (Fig. 6.4). Each natural and bioengineered vibrissa follicle contained 500~1000 DP cells. Thus, these findings provide new insights regarding the regulation of hair properties and strongly suggest that these characteristics can be properly restored by cell processing for organ regeneration and by transplantation of the bioengineered hair follicle germ.

It is well known that hair follicle organ-inductive epithelial and mesenchymal stem cells provide a source of differentiated hair follicle cells that enable hair cycling to occur over the lifetime of a mammal. It is also essential to rearrange these various stem cells and their niches in the bioengineered follicle to reproduce enduring hair cycles. The bioengineered follicles that were reproduced using an organ germ method and intracutaneous transplantation could reconstruct various niches, such as CD34- and CD49f-positive epithelial stem cells and SOX2-positive vibrissa

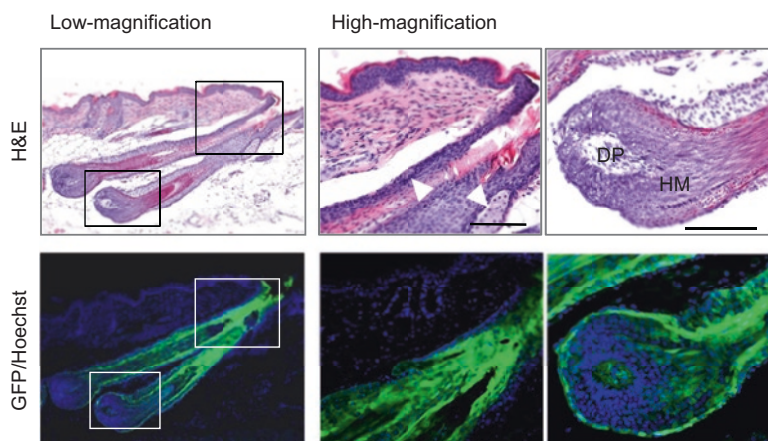


Fig. 6.4 Structural features of the bioengineered hair follicle. The GFP-labeled bioengineered vibrissa follicles were stained with H&E (*upper panels*) and Hoechst (*lower panels*). The *boxed areas* in the low-magnification H&E and fluorescent panel are shown at a higher magnification in the *center and right panels*. The *arrowhead* indicates a sebaceous gland. Scale bars, 100 μ m

mesenchymal stem cells and progenitors. To evaluate the functional restoration of the hair follicle stem cells and their niches in the bioengineered hair follicle, the bioengineered hair cycles were analyzed for 80 days. The bioengineered pelage and vibrissa follicles repeated the hair cycle at least 3 times during the 80-day period, and there were no significant differences in the hair cycle periods between the natural and bioengineered follicles. These results indicated that the bioengineered hair follicle could undergo proper hair cycles according to the cell types of origin. The bioengineered pelage and vibrissa follicles have been suggested to reproduce these hair cycles, which are maintained by stem cells and provide a stem cell niche.

Hair shaft pigmentation is provided by melanocyte in the hair matrix, and melanocyte stem cells are maintained in the sub-bulge region of vibrissa hair follicles during anagen (Nishimura et al. 2002, 2005) (Fig. 6.5). Melanocyte progenitor cells, which are widely distributed between the ORS of the variable region and the proximal region of the hair matrix, can proliferate to provide melanogenic cells (Nishimura et al. 2002, 2005). The unpigmented bioengineered vibrissa hair follicle lacks melanocyte stem/progenitor cells, resulting in white hair shafts (Fig. 6.3c and 6.5). The addition of the proximal end of hair matrix cells, which contain melanocyte progenitor or stem cells, resulted in pigmented bioengineered vibrissae (Fig. 6.5). These results indicated that various follicle stem cells and their niches were successfully rearranged in the bioengineered hair follicles using appropriate epithelial and mesenchymal cell populations.

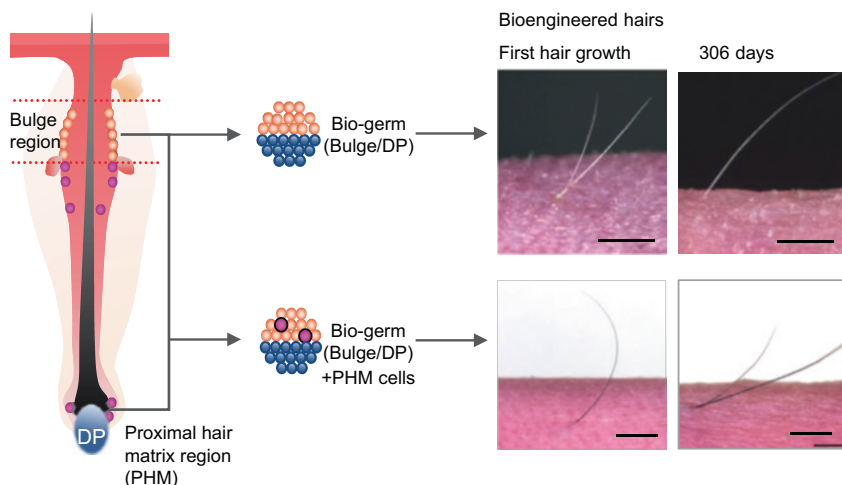


Fig. 6.5 Hair pigmentation of the bioengineered vibrissae by cell combinations with bioengineered vibrissa follicle cell components. The bioengineered vibrissa follicle germ, which was reconstituted between the bulge epithelial stem cells and the primary cultured DP cells (*upper*), was combined with the proximal region of the hair matrix (+PHM; *lower*). Unpigmented and pigmented bioengineered hairs at the first growth phase and at 306 days after transplantation are shown in *right* photographs, respectively. Bars, 1 mm

6.7.3 *Reproduction of the Cooperative Function of the Bioengineered Hair Follicle*

The peripheral nervous system has essential roles in organ function and the perception of noxious stimuli, such as pain and mechanical stress (Grant et al. 2009; Peters et al. 2006; Jahoda and Christiano 2011). Restoration of the nervous system is thus a critical issue that must be addressed by organ replacement regenerative therapy (Toyoshima et al. 2012; Asakawa et al. 2012; Tezuka et al. 2016). In the hair follicle, the follicles, pelage, and vibrissae achieve piloerection using the surrounding arrector pili muscle through activation of the sympathetic nerves and also function as a sensory organ (Peters et al. 2001, 2006; Sato et al. 2012). Toyoshima et al. also demonstrated that the nerve fibers and muscles can connect autonomously to the pelage and vibrissa follicle and that the bioengineered follicles exhibit piloerection. These results suggest that the transplantation of a bioengineered hair follicle germ can restore natural hair function and reestablish the cooperation between the follicle and the surrounding recipient muscles and nerves (Toyoshima et al. 2012; Asakawa et al. 2012; Tezuka et al. 2016) (Fig. 6.6). Thus, transplantation of the bioengineered hair follicle germ may be applicable for the future surgical treatment of alopecia (Toyoshima et al. 2012).

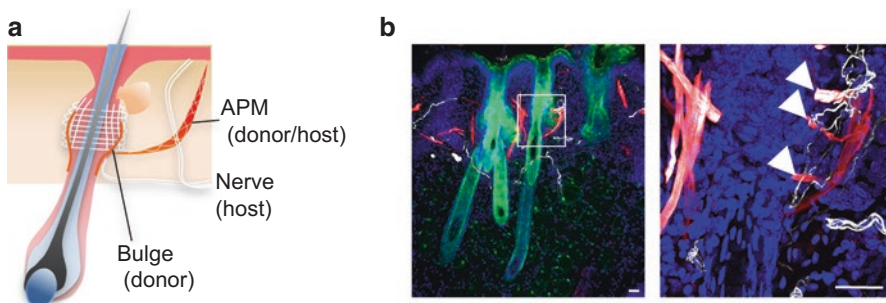


Fig. 6.6 Analyses of connections to surrounding tissues and the piloerection capability of bioengineered hair follicles. **(a)** Schematic representation of the connections of the bioengineered hair follicles with surrounding tissues in adult skin. **(b)** The connections of the follicles of a EGFP-labeled bioengineered pelage to arrector pili muscles (*red signal*) and nerve fibers (*white signal*) were analyzed by immunohistochemical staining. The boxed areas in the *left panel* are shown at a higher magnification on the *right*. The *arrowheads* indicate the muscle and nerve fibers connected to the pelage follicles. Scale bars, 100 μ m at low magnification and 50 μ m at high magnification

6.7.4 Human Bioengineered Hair Follicle

One of the ultimate goals of research investigating hair follicle regeneration is the clinical translation for the treatment of hair loss and various hair-associated disorders. A number of researchers have reported the hair follicle-forming potential of dissected tissues and cultured cells derived from human biopsies; however, no one has provided conclusive evidence for the regeneration of a fully functional human hair follicle. The human bioengineered hair follicle germ, which consists of dissociated bulge region-derived epithelial cells and scalp hair follicle-derived intact DPs in an androgenetic alopecia patient, could regenerate the human hair follicle and support the growth of a pigmented hair shaft in the intracutaneous transplantation area of nude mice. This bioengineered hair follicle was composed of correct structures, consisting minimally of an infundibulum and sebaceous gland, hair shaft, inner root sheath, ORS, hair matrix, and DP in the hair bulb structure, which were confirmed to be of human origin (Toyoshima et al. 2012). This result indicates that the bioengineered hair follicle germ method is applicable to human-derived cells and contributes to future developments in hair follicle regenerative medicine (Toyoshima et al. 2012).

6.8 Future Perspectives for the Hair Follicle

To achieve hair follicle regeneration using stem cells in the adult hair follicle, defining the regeneration of the various stem cells and their niches is considered essential. Based on the preceding studies, we can utilize the extensive knowledge of various types of follicular stem cells in the hair follicle (Chuong et al. 2007).

The bioengineered hair follicle germ can develop into a fully functional hair follicle through the rearrangement of various stem cells and their niches (Toyoshima et al. 2012). The dissected bioengineered hair follicles can also restore fully physiological hair functions in adult skin via ectopic transplantation of the bioengineered hair follicle germs (Toyoshima et al. 2012). Future studies of in vitro culture systems that can reproduce bioengineered hair follicles from bioengineered hair follicle germs permit the promotion of therapeutic systems such as FUT in the clinic.

To successfully provide clinically useful and effective hair follicle regenerative therapy, several issues persist, such as the optimization of human hair follicle-derived stem cell sources and in vitro expansion for clinical applications (Ohyama 2007). There are significant variations in follicular stem cell marker expression among species, hair types, skin surface regions, and individuals, although the clinical definition of useful stem cell markers is maintained (Ohyama 2007). There is no way to satisfactorily in vitro propagate human follicular stem cells using clinically available culture systems. The organ germ method and functional hair regeneration assay assist the development of a high-throughput system, which would facilitate techniques leading to the clinical application of hair follicle regenerative therapy.

Recent biological and technical innovations have achieved substantial progress in the development of a novel therapeutic model for hair follicle regenerative therapy for alopecia and organ replacement regenerative therapy. Further studies focused on the optimization of human hair follicle-derived stem cell sources for clinical applications of stem cell niches will contribute to the development of hair regenerative therapy as a prominent class of organ replacement regenerative therapy in the future.

Acknowledgments This work was partially supported by Organ Technologies, Inc.

Conflict of Interest K. Toyoshima and T. Tsuji have no competing interests.

References

- Akiyama M, Smith LT, Shimizu HJ (2000) Changing patterns of localization of putative stem cells in developing human hair follicles. *Invest Dermatol* 114:321–327
- Andl T, Reddy ST, Gaddapara T, Millar SE (2002) WNT Signals Are Required for the Initiation of Hair Follicle Development. *Dev Cell* 2:643–653
- Asakawa K, Toyoshima K-E, Ishibashi N, Tobe H, Iwadata A, Kanayama T, Hasegawa T, Nakao K, Toki H, Noguchi S, Ogawa M, Sato A, Tsuji T (2012) Hair organ regeneration via the bioengineered hair follicular unit transplantation. *Sci Rep* 2:424. doi:[10.1038/srep00424](https://doi.org/10.1038/srep00424)
- Atit R, Sgaier SK, Mohamed OA et al (2006) β -Catenin activation is necessary and sufficient to specify the dorsal dermal fate in the mouse. *Dev Biol* 296:164–176
- Benitah SA, Frye M (2012) Stem cells in ectodermal development. *J Mol Med* 90:783–790
- Botchkarev VA, Kishimoto J (2003) Molecular Control of Epithelial-Mesenchymal Interactions During Hair Follicle Cycling. *J Invest Dermatol Symp Proc* 8:46–55

- Carlsen RA (1974) Human fetal hair follicles: the mesenchymal component. *J Invest Dermatol* 63:206–211
- Chuong CM, Cotsarelis G, Stenn K (2007) Defining hair follicles in the age of stem cell bioengineering. *J Invest Dermatol* 127:2098–2100
- Claudinet S, Nicolas M, Oshima H, Rochat A, Barrandon Y (2005a) Long-term renewal of hair follicles from clonogenic multipotent stem cells. *Proc Natl Acad Sci USA* 102:14677–14682
- Claudinet S, Nicolas M, Oshima H, Rochat A, Barrandon Y (2005b) Long-term renewal of hair follicles from clonogenic multipotent stem cells. *Proc Natl Acad Sci U S A* 102:14677–14682
- 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
- Driskell RR, Giangreco A, Jensen KB, Mulder KW, Watt FM (2009) Sox2- positive dermal papilla cells specify hair follicle type in mammalian epidermis. *Development* 136:2815–2823
- Ehama R, Ishimatsu-Tsuji Y, Iriyama S, Ideta R, Soma T, Yano K, Kawasaki C, Suzuki S, Shirakata Y, Hashimoto K, Kishimoto J (2007) Hair follicle regeneration using grafted rodent and human cells. *J Invest Dermatol* 127:2106–2115
- Festa E, Fretz J, Berry R, Schmidt B, Rodeheffer M, Horowitz M, Horsley V (2011) Adipocyte lineage cells contribute to the skin stem cell niche to drive hair cycling. *Cell* 146:761–771
- Fu J, Hsu W (2013) Epidermal Wnt controls hair follicle induction by orchestrating dynamic signaling crosstalk between the epidermis and dermis. *J Invest Dermatol* 133:890–898
- Fuchs E (2007) Scratching the surface of skin development. *Nature* 445:834–842
- Fujiwara H et al (2011) The basement membrane of hair follicle stem cells is a muscle cell niche. *Cell* 144:577–589
- Grant RA, Mitchinson B, Fox CW, Prescott TJ (2009) Active touch sensing in the rat: anticipatory and regulatory control of whisker movements during surface exploration. *J Neurophysiol* 101:862–874
- Greco V et al (2009) A two-step mechanism for stem cell activation during hair regeneration. *Cell Stem Cell* 4:155–169
- Hardy MH (1949) The development of mouse hair in vitro with some observations on pigmentation. *J Anat* 83(4):364–384
- Hardy MH (1992) The secret life of the hair follicle. *Trends Genet* 8:55–61
- Hibberts NA, Howell AE, Randall VA (1998) Balding hair follicle dermal papilla cells contain higher levels of androgen receptors than those from non-balding scalp. *Journal of Endocrinology* 6:559–565
- Hojiro O (1972) Fine structure of the mouse hair follicle. *J Electron Microsc* 21:127–138
- Horne KA, Jahoda CA, Oliver RF (1986) Whisker growth induced by implantation of cultured vibrissa dermal papilla cells in the adult rat. *J Embryol Exp Morphol* 97:111–124
- Hsu YC, Li L, Fuchs E (2014) Emerging interactions between skin stem cells and their niches. *Nat Med* 20(8):847–856
- Ihara S, Watanabe M, Nagao E, Shioya N (1991) Formation of hair follicles from a single-cell suspension of embryonic rat skin by a two-step procedure in vitro. *Cell Tissue Res* 266:65–73
- Iida M, Ihara S, Matsuzaki T (2007) Hair cycle-dependent changes of alkaline phosphatase activity in the mesenchyme and epithelium in mouse vibrissal follicles. *Dev Growth Differ* 49:185–195
- Ikeda E et al (2009) Fully functional bioengineered tooth replacement as an organ replacement therapy. *Proc Natl Acad Sci USA* 106:13475–13480
- Jahoda CA, Christiano AM (2011) Niche Crosstalk: Intercellular Signals at the Hair Follicle. *Cell* 146:678–681
- Jahoda CA, Horne KA, Oliver RF (1984) Induction of hair growth by implantation of cultured dermal papilla cells. *Nature* 311:560–562
- Jahoda CA, Reynolds AJ (2001) Hair follicle dermal sheath cells: unsung participants in wound healing. *Lancet* 358:1445–1448

- Jahoda CA, Whitehouse J, Reynolds AJ, Hole N (2003) Hair follicle dermal cells differentiate into adipogenic and osteogenic lineages. *Exp Dermatol* 12:849–859
- Waters JM, Richardson GD, Jahoda CAB (2007) Hair follicle stem cells. *Seminars in Cell & Developmental Biology* 18:245–254
- Jensen KB et al (2009) Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis. *Cell Stem Cell* 4:427–439
- Kishimoto J et al (1999) Selective activation of the versican promoter by epithelial-mesenchymal interactions during hair follicle development. *Proc. Natl Acad Sci USA* 96:7336–7341
- Lavker RM, Sun TT, Oshima H, Barrandon Y, Akiyama M, Ferraris C, Chevalier G, Favier B, Jahoda CA, Dhoubailly D, Panteleyev AA, Christiano AM (2003) Hair follicle stem cells. *J Invest Dermatol Symp Proc* 8:28–38
- Lee LF, Chuong CM (2009) Building complex tissues: high-throughput screening for molecules required in hair engineering. *J Invest Dermatol* 129:815–817
- Lichti U, Anders J, Yuspa SH (2008) Isolation and short-term culture of primary keratinocytes, hair follicle populations and dermal cells from newborn mice and keratinocytes from adult mice for in vitro analysis and for graying to immunodeficient mice. *Nat Protoc* 3:799–810
- Lim X, Nusse R (2013) Wnt Signaling in Skin Development, Homeostasis, and Disease. *Cold Spring Harb Perspect Biol* 5:a08029
- Mannik J, Alzayady K, Ghazizadeh S (2010) Regeneration of multilineage skin epithelia by differentiated keratinocytes. *J Invest Dermatol* 130:388–397
- Millar SE (2002) Molecular mechanisms regulating hair follicle development. *J Invest Dermatol* 116:216–8225
- Morris RJ et al (2004) Capturing and profiling adult hair follicle stem cells. *Nat Biotechnol* 22:411–417
- Mounsey AL, Reed SW (2009) Diagnosing and treating hair loss. *Am Fam Physician* 80:356–362
- Nakao K et al (2007) The development of a bioengineered organ germ method. *Nat Methods* 4:227–230
- Nishimura EK et al (2002) Dominant role of the niche in melanocyte stem-cell fate determination. *Nature* 416:854–860
- Nishimura EK, Granter SR, Fisher DE (2005) Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche. *Science* 4:720–724
- Ohtola J, Myers J, Akhtar-Zaidi B et al (2008) β -catenin has sequential roles in the survival and specification of ventral dermis. *Development* 135:2321–2329
- Ohyama M (2007) Hair follicle bulge: a fascinating reservoir of epithelial stem cells. *Journal of Dermatological Science* 46:81–89
- Ohyama M, Terunuma A, Tock CL, Radonovich MF, Pise-Masison CA, Hopping SB, Brady JN, Udey MC, Vogel JC (2006) Characterization and isolation of stem cell-enriched human hair follicle bulge cells. *J Clin Invest* 116(1):249–60
- 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
- Osada A, Iwabuchi T, Kishimoto J, Hamazaki TS, Okochi H (2007) Long-term culture of mouse vibrissa dermal papilla cells and de novo hair follicle induction. *Tissue Eng* 13:975–982
- Oshima H, Rochat A, Kedzia C, Kobayashi K, Barrandon Y (2001) Morphogenesis and renewal of hair follicles from adult multipotent stem cells. *Cell* 104:233–245
- Ouji Y, Nakamura-Uchiyama F, Yoshikawa M (2013) Canonical Wnts, specifically Wnt-10b, show ability to maintain dermal papilla cells. *Biochem Biophys Res Commun* 438:493–499
- Paus R, Muller-Rover S, Veen C et al (1999) A comprehensive guide for the recognition and classification of distinct stages of hair follicle morphogenesis. *J Invest Dermatol* 113:523–532
- Peters EM, Botchkarev VA, Botchkareva NV, Tobin DJ, Paus R (2001) Hair-cycle-associated remodeling of the peptidergic innervation of murine skin, and hair growth modulation by neuropeptides. *J Invest Dermatol* 116:236–245

- Peters EMJ, Arck PC, Paus R (2006) Hair growth inhibition by psychoemotional stress: a mouse model for neural mechanisms in hair growth control. *Exp Dermatol* 15:1–13
- Plikus MV, Mayer JA, de la Cruz D, Baker RE, Maini PK, Maxson R, Chuong CM (2008) Cyclic dermal BMP signalling regulates stem cell activation during hair regeneration. *Nature* 451:340–344
- Polykandriotis E, Popescu LM, Horch RE (2010) Regenerative medicine: then and now an update of recent history into future possibilities. *J Cell Mol Med* 14:2350–2358
- Qi J, Garza LA (2014) An overview of Alopecias. *Cold Spring Harb Perspect Med* 4:a013615
- Rahmani W, Abbasi S, Hagner A et al (2015) Hair follicle dermal stem cells regenerate the dermal sheath, repopulate the dermal papilla, and modulate hair type. *Dev Cell* 31:543–558
- Ramos PM, Miot HA (2015) Female pattern hair loss: a clinical and pathophysiological review. *An Bras Dermatol* 90:529–543
- Randall VA, Thornton MJ, Messenger AG et al (1993) Brinklow BR: hormones and hair growth: variations in androgen receptor content of dermal papilla cells cultured from human and red deer (*Cervus elaphus*) hair follicles. *J Invest Dermatol* 101:114S–120S
- Reynolds AJ, Jahoda CA (1991) Hair follicle stem cells? A distinct germinative epidermal cell population is activated in vitro by the presence of hair dermal papilla cells. *Journal of Cell Science* 99:373–385
- Reynolds AJ, Jahoda CA (1996) Hair matrix germinative epidermal cells confer follicle-inducing capabilities on dermal sheath and high passage papilla cells. *Development* 122:3085–3094
- Reynolds AJ, Lawrence C, Cserhalmi-Friedman PB, Christiano AM, Jahoda CA (1999) Transgender induction of hair follicles. *Nature* 402(6757):33–34
- Rivera-Gonzalez G, Shook B, Horsley V (2014) Adipocytes in skin health and disease. *Cold Spring Harb Perspect Med* 4:a015271
- Ross SE, Hemati N, Longo KA et al (2000) Inhibition of adipogenesis by Wnt signaling. *Science* 289:950–953
- Sasai Y (2013a) Cytosystems dynamics in self-organization of tissue architecture. *Nature* 493:318–326
- Sasai Y (2013b) Next-generation regenerative medicine: organogenesis from stem cells in 3D culture. *Cell Stem Cell* 12:520–530
- Sato A et al (2012) Single follicular unit transplantation reconstructs arrector pili muscle- and nerve-connections and restores functional hair follicle piloerection in preparing. *J Dermatol* 39:1–6
- Schneider MR, Schmidt-Ullrich R, Paus R (2009) The hair follicle as a dynamic miniorgan. *Curr Biol* 19(3):R132–R142
- Sperling LC (2001) Scarring alopecia and the dermatopathologist. *J Cutan Pathol* 28:333–342
- Stenn K et al (2007) Bioengineering the hair follicle. *Organogenesis* 3:6–13
- Stenn KS, Paus R (2001) Controls of hair follicle cycling. *Physiol Rev* 81:449–494
- Takeda A, Matsuhashi S, Shioya N, Ihara S (1998) Histodifferentiation of hair follicles in grafting of cell aggregates obtained by rotation culture of embryonic rat skin. *Scand J Plast Reconstr Surg Hand Surg* 32:359–364
- Tezuka K, Toyoshima KE, Tsuji T (2016) Hair follicle regeneration by transplantation of a bioengineered hair follicle germ. *Methods Mol Biol* 1453:71–84
- Thornton MJ, Kato S, Hibberts NA, Brinklow BR, Loudon AS, Randall VA (1996) Ability to culture dermal papilla cells from red deer (*Cervus elaphus*) hair follicles with differing hormonal responses in vivo offers a new model for studying the control of hair follicle biology. *J Exp Zool* 275:452–458
- Toyoshima KE, Asakawa K, Ishibashi N et al (2012) Fully functional hair follicle regeneration through the rearrangement of stem cells and their niches. *Nat Commun* 3:784
- Unger W, Shapiro R, Unger MA, Unger U (2010) Hair transplantation, 5th edn. Informa Healthcare UK, New York

- Vidal VP, Chaboissier MC, Lützkendorf S, Cotsarelis G, Mill P, Hui CC, Ortonne N, Ortonne JP, Schedl A (2005) Sox9 is essential for outer root sheath differentiation and the formation of the hair stem cell compartment. *Curr Biol* 15:1340–1351
- Wang X, Tredget EE, Wu Y (2012) Dynamic signals for hair follicle development and regeneration. *Stem Cells Dev* 21(1):7–18
- Weinberg WC et al (1993) Reconstitution of hair follicle development in vivo: determination of follicle formation, hair growth, and hair quality by dermal cells. *J Invest Dermatol* 100:229–236
- Zheng Y et al (2005) Organogenesis from dissociated cells: generation of mature cycling hair follicles from skin-derived cells. *J Invest Dermatol* 124:867–876