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Bioengineering the hair follicle: fringe benefits of stem cell technology

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Recent advances in epithelial stem cell biology have resulted in the isolation of hair follicle stem cells, which generate hair follicles when injected into immunodeficient mice. These isolated hair follicle epithelial stem cells must be combined with 'inductive' dermal cells to produce new hair follicles. The advent of techniques for cultivating inductive dermal cells and competent epithelial stem cells creates the opportunity to bioengineer hair follicles for the treatment of hair loss.

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Introduction

Because of the critical role hair plays in human non-verbal communication, when hair growth diminishes the affected individual invariably demands help. The ultimate therapy, of course, is to restore or regenerate new, healthy, cycling hair follicles. Until very recently, medicine was unable to offer any valid treatments to these patients. In fact, the false claims in the past millennia have given the term 'hair growth treatments' a very negative, if not derisive, connotation. In the late twentieth century, several drugs were marketed that, however modestly and inconsistently, did stimulate hair growth. These, serendipitously discovered, first-generation hair growth drugs — minoxidil, finasteride and latanoprost — are landmarks because they give us encouragement and justification that we can indeed affect hair appearance medically [1]. Nevertheless, their effects fall short of the ultimate goal to generate new hair follicles in bald scalp.

Clues for bioengineering hair follicles can be gleaned from what we know about normal hair follicular morphogenesis and growth (Figure 1) [2–4]. In fetal skin, hair

follicles develop from two major cell types — epithelium and mesenchyme — and crosstalk between these two cell populations is critical. As no new hair follicles form after birth, bioengineering new hair follicles may seem unduly challenging. However, unlike other organs, each hair follicle normally regenerates itself cyclically in a manner that recapitulates embryonic hair follicle development (Figure 1). In the adult, the lower hair follicle reforms itself with each new hair cycle by the interaction of the epithelial stem cells in the bulge with adjacent mesenchymally derived dermal papilla cells. Thus, the adult follicle possesses an innate ability to regenerate its hair-producing apparatus using cells located in specific follicular niches.

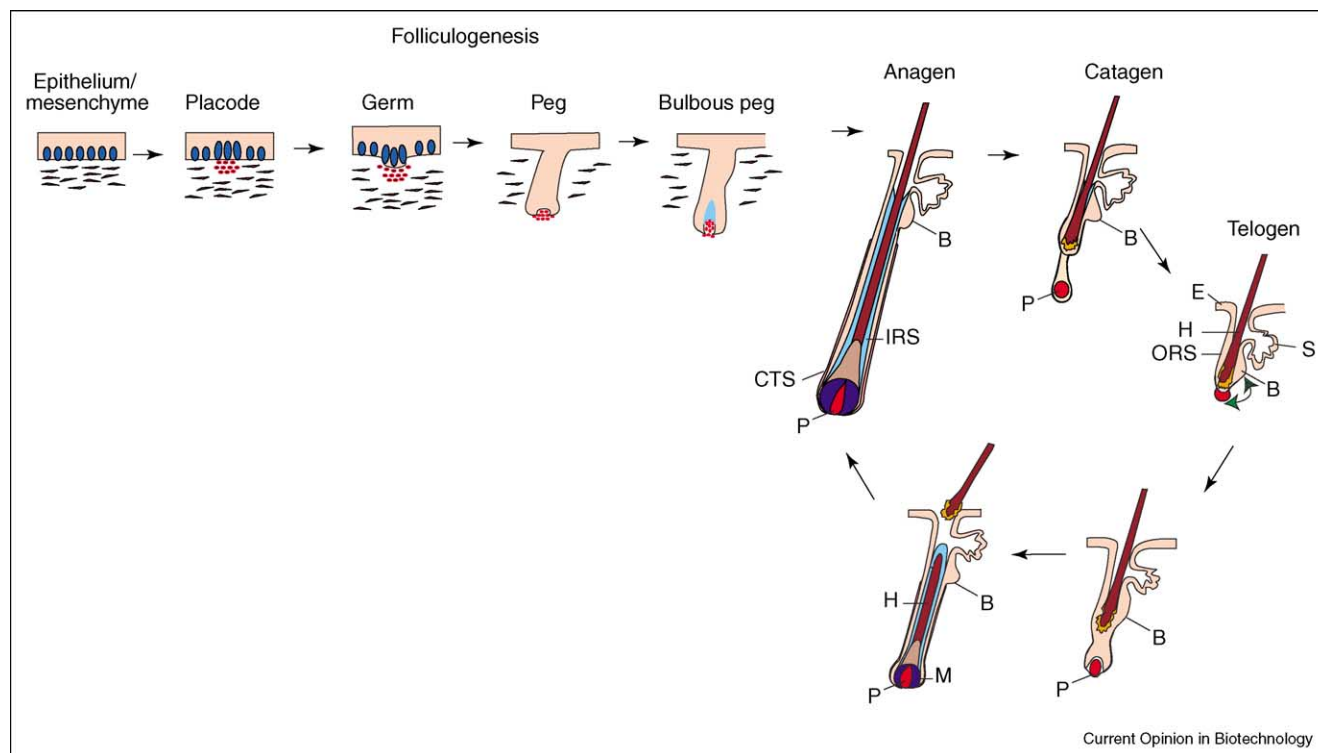
In this review, our goal is to acquaint the reader with the hair follicle, and the potential to generate new hair follicles through tissue engineering. The review is not meant to cover the molecular signals involved in hair follicle development and cycling (as in [4]). We focus on the implications of recent advances in hair follicle stem cell isolation and in propagation of these cells along with inductive dermal cells. We provide two possible scenarios for using dermal inductive cells to generate new follicles in bald scalp.

Hair follicle stem cells

The epithelial stem cell niche is located in the hair follicle bulge, which is part of the outer root sheath that is in continuity with the interfollicular epidermis and sebaceous gland (Figure 1). Because of their quiescent nature and strategic location, bulge cells were postulated to be hair follicle stem cells in both mice [5] and men [6]. This hypothesis led to multiple studies that tested the notion that bulge stem cells in adult mice are multipotent and regenerate a lower hair follicle during normal cycling [7,8]. Lineage analysis definitively demonstrated that bulge cells give rise to all of the epithelial cell types in the normal regenerating lower follicle during anagen (i.e. the growth phase; see Figure 1), in addition to the sebaceous gland and overlying epidermis [9•].

Recently, workers addressed the therapeutically relevant question of whether isolated hair follicle bulge epithelial cells can generate new hair follicles [9•,10•]. This became possible with the identification of bulge cell markers, CD34 [11•] and cytokeratin 15 promoter activity [12]. It was shown that bulge cells do indeed retain their 'competence' for generating new hair follicles when removed from their environment or niche. This feat was only possible, however, when bulge cells were

Figure 1



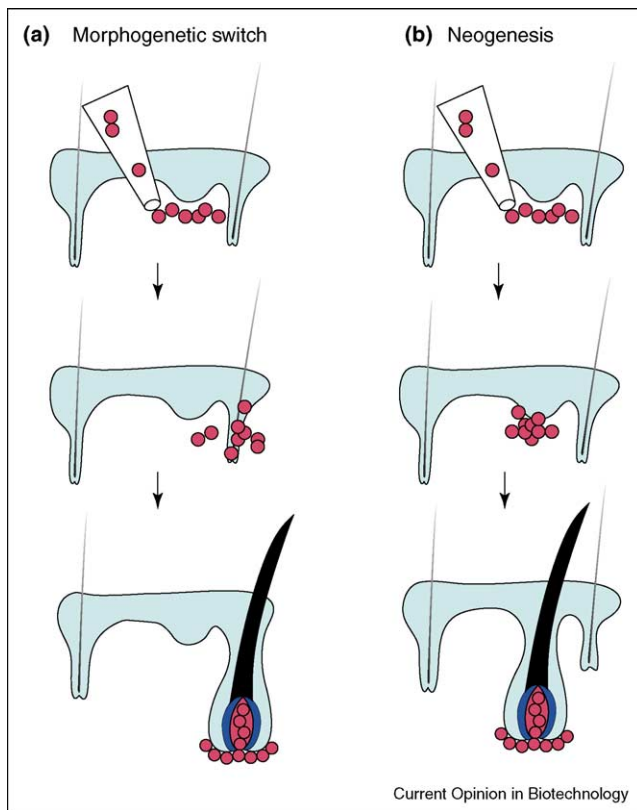
Schematic illustrating the formation of hair follicles in the fetus and its cycling transformations in the adult. In the fetus, a thickening forms at one focus of the primitive epithelium to form a placode. Below the placode dermal cells aggregate and thereafter the epithelium grows down as a finger to produce the multilayer, shaft-producing hair follicle. In the adult, three phases of the growth cycle are recognized, a growth phase (anagen), a regressing phase (catagen), and a resting phase (telogen). It is the lower follicle that regenerates at the beginning of each cycle by utilizing intimate and powerful epithelial-mesenchymal interactions of the stem cells in the bulge (B) and the inductive mesenchymal cells of the papilla (P). CTS, connective tissue sheath; E, epidermis; H, hair shaft; IRS, inner root sheath; M, hair matrix; ORS, outer root sheath; S, sebaceous gland.

combined with 'inductive' dermal cells obtained from neonatal dermis. Although hair follicle bulge cells appear to be the only epithelial progenitors of the lower follicle in normal skin, this competence to form follicles is not exclusive to bulge cells. Interfollicular epidermal cells also retain some capacity to generate new hair follicles both *in situ* and after isolation; however, the efficiency of bulge cells to form follicles appears greater, suggesting that starting with a pure population of stem cells would increase the effectiveness of cell-based therapies. Additional studies suggest that other epithelia [13], cultured epithelial cells [10^{••},14], corneal epithelium [15] or amnion [16] also have the capacity to generate hair follicles when juxtaposed with inductive dermal cells. In fact, numerous types of stem cells, including embryonic, neural [17], and mesenchymal bone marrow [18] all have the capacity to form skin and hair when introduced into blastocysts. This raises the intriguing possibility that these other types of stem cell could be coaxed into forming hair follicles by the proper inductive cells.

Inductive dermal cells

Many investigators found that a follicular dermal papilla dissected from the base of an adult anagen hair follicle — either fresh or after tissue culture expansion — could induce new hair follicle formation in rodents if placed in proximity to the epithelium [13,14,19,20,21^{••},22[•],23–26]. Perhaps the most dramatic demonstration of the inductive ability of the dermal portion of the follicle comes from the work of Reynolds and colleagues [27] who transplanted the connective tissue sheath, a structure contiguous to the papilla (Figure 1), from the scalp of a man to the forearm of a woman. Remarkably, 3–5 weeks later new hair follicles were found at the site of implantation. The mesenchymal portion of the 'new' follicle arose from the donor male follicle, but the origin of the epithelial portion was not clear. An existing small follicle may have converted to a large hair follicle by the implanted sheath cells (Figure 2a; morphogenetic switch), or the interfollicular epidermis in the recipient's arm may have generated a hair follicle *de novo* in response to the inductive dermal signals (Figure 2b; neogenesis model).

Figure 2



New hair follicle formation following the delivery of trichogenic cells to the dermis is believed to occur by one of two mechanisms: the morphogenetic switch or neogenesis. **(a)** In the morphogenetic switch model, the injected trichogenic cells (red) are incorporated into small pre-existing follicles of the skin and the donor cells induce the recipient small hair follicles to become large. **(b)** In the neogenesis model, after trichogenic cells are injected into the skin the inductive cells interact with the recipient epidermis to generate a follicle in a pattern very similar to that seen in the fetus. By this mechanism true new follicle formation occurs.

Whatever the mechanism, the powerful inductive ability of the follicular dermal cells is incontrovertible.

One approach to hair follicle cell-based therapy would entail removing a small number of hair follicles, isolating competent and/or inductive cells from them, and then expanding those cells *ex vivo* while maintaining their special ability to generate new hair follicles. Clearly, cell culture conditions that maintain the inductive ability of dermal follicular cells and the competence of hair follicle epithelial cells are necessary before any type of cell-based therapy for alopecia can be developed. As early studies showed that the inductive property of dermal cells wanes with time *in vitro*, much research has focused on maintaining the trichogenic properties of hair follicle cells in culture. Seminal work by Kishimoto *et al.* [21^{••}] showed that the Wnt pathway is necessary for maintaining the inductive ability of cultured papilla cells; this finding

allowed the generation of large numbers of inductive dermal cells from a small number of donor hair follicles. Similarly for epithelial cells, Blanpain *et al.* [10^{••}] showed in mouse that cloned bulge cells can be amplified in culture using standard techniques with feeder cells, and then used in reconstitution assays to regenerate new hair follicles when combined with neonatal dermal cells. However, whether non-bulge keratinocytes possess similar properties was not reported. Future studies addressing these issues, especially in human systems, are necessary.

Approaches for bioengineering the hair follicle

Thus, for bioengineering the hair follicle, one could start with dermal elements from dissociated follicles with or without competent cells from the follicle or other epithelial sources. The number of dissociated cells would be expanded in culture and then dermal cells alone, or in combination with competent epithelial cells, re-introduced to the alopecic scalp. Previous studies have shown that starting with correctly placed inducer dermal cells will result in new follicle formation [20,22[•],28]. Moreover, starting with a combination of dissociated [29], or aggregated [30–33], trichogenic epithelial and dermal cells has also proven to be an efficient way of producing new hair follicles.

First attempts at cell-based approaches for treating alopecia are likely to use autologous tissue for bioengineering hair follicles to avoid immune rejection of the donor cells. However, the intriguing possibility that heterologous (allogeneic) hair follicle tissue could be developed for tissue transplantation exists, based on the concept that the hair follicle is an immune-privileged site that does not express MHC (major histocompatibility complex) class I antigens [9^{••},34]. As dermal hair follicle tissue has already been transplanted from one individual to another without evidence of rejection [27], this possibility may not be as implausible as originally thought. Nevertheless, the safety testing and regulatory hurdles for this type of approach would require enormous financial resources.

Another possible approach for bioengineering hair follicles involves actually forming hair follicles as mini organs *in vitro*, and then transplanting the newly generated follicles back to the alopecic scalp. This sort of approach would require a much more complicated cell culture system involving three-dimensional matrices, perhaps embedded with appropriate growth factors, to allow both dermal and epidermal cells to differentiate towards a normal hair follicle. The bioengineering literature reports extensive experience with biocompatible materials that might be exploited here [35,36]. The advantages to this approach include possible genetic manipulation of hair follicle cells *ex vivo*, and facilitation of the surgical placement of the new hair follicle in the proper orientation.

Challenges associated with bioengineering the hair follicle

Major challenges that need to be addressed with any type of cell-based treatment for alopecia include the efficiency of hair follicle formation and the choice of cell type. For example, how many new hair follicles can be generated from a given number of donated hair follicles? Clearly, the ratio of new hair follicles to donor hair follicles must be as high as possible to produce a clinically successful product. Other cell types, such as melanocytes and Merkel cells, normally reside in the follicle. Will these cells develop or be recruited to the new follicle? In addition, how can we be certain that the follicles formed will cycle? On this latter point, we have evidence that follicles formed from dissociated trichogenic cells will cycle repeatedly with the same phase as their follicles of origin [37]. Moreover, the follicles formed show the same morphology as the follicles from which the dermal cells were derived [37,38]. An interesting aspect of new organ formation is raised by bone marrow transplant studies which show that cells arising from the bone marrow, which enter the circulation, may contribute to a wound or reparative cellular response [39,40,41]. It is notable that such cells will contribute to skin and hair follicle repair [42]. Would a regenerating system also attract cells that might affect (enhance or inhibit) organogenesis overall? Other challenges include how to achieve an appropriate patterning and angling of the hair follicle.

Much progress has been made in understanding the molecular pathways activated during hair follicle embryogenesis and cycling [3,4]. Eventually, this understanding should lead to the generation of new pharmaceutical agents that specifically target these pathways [2]. However, the complex timing and myriad gene expression changes required for orchestration of hair follicle development and cycling are likely to preclude a simple pharmaceutical approach to the treatment of advanced alopecia. By taking advantage of cell types that 'know' how to form a hair follicle, we assume cell-based therapies will arrive in the clinic sooner than the purely molecular approach.

Although the time is ripe to successfully engineer new hair follicles, we recognize a lesson that workers in the fields of stem cell biology and biotechnology have painfully learned — laboratory animal studies might not translate to humans. Very few folliculoneogenesis studies have been conducted in humans. Our greatest successes have been made with animal models, and for hair growth (mammalian systems) that model has been the mouse. Intermediate studies could involve testing cell-based treatments on human skin grafted to immunodeficient mice [43]. The ultimate test will be the clinical study.

Conclusions

In summary, we believe that the creation of new hair follicles for the treatment of alopecia through tissue

engineering is achievable. The hair follicle reforms itself by means of interactions between competent epithelial stem cells and powerfully inductive dermal cells during its growth cycle. A product designed to form new hair follicles could be conceived to have the competent epithelial cells, the inductive dermal cells or a combination of both, delivered to the correct layer of the dermis. Although in this review we have examined in some detail the elements of hair follicle engineering, we believe our efforts embrace the same engineering challenges that other organ systems will face, such as the eye, liver, pancreatic islet, finger, and so on. Because of its inherent regenerative properties and the nature of the market demand, the hair follicle is likely to be the first organ regeneration system to successfully reach the clinic.

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