A Simplified Procedure to Reconstitute Hair-Producing Skin

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One of the major objectives of tissue engineering is to reconstitute skin from stem cells. This requires multipotent skin stem cells and the ability to guide these cells to form a piece of skin with proper architecture and skin appendages. Based on previous progress, we develop a simplified procedure that can be useful for large-scale screening of factors that can modulate the hair formation ability of candidate cells. Newborn mouse cells are used. Dissociated epidermal and dermal cells in high-density suspension are allowed to reconstitute *in vitro* to generate its own matrix, or seeded into a scaffold-like matrix already used clinically. These cells self-organize and form a reconstituted skin with proper proportions and topological organization of different components. Large numbers of hair follicles form. The cellular and molecular events are characterized, showing a distinct but parallel morphogenetic process compared to those occurring in embryonic development. The formed hair follicles can cycle and regenerate and the reconstituted skin can heal after injury. The skins are in good condition 1 year after transplant. This procedure enables flexible size and shape of the reconstituted skin, so clinical applications can be envisioned for the future when large numbers of multipotential skin stem cells become available.

Introduction

THE ABILITY TO RECONSTITUTE adult skin with functional skin appendages has long been a major clinical objective for dermatologists and surgeons. To this end, Lichti *et al.*¹ was the first to show that it is possible to use dissociated hair precursor cells to have *de novo* formation of hair follicles *in vivo*. Their protocol is now widely used to mix dissociated multipotential dermal and epidermal stem cells from newborn mouse skin to form hairs. This assay and its modifications have been used to test the ability of hair bulge stem cells from adult mice to form hairs.^{2,3}

Lichti's chamber procedure^{1,4} is an achievement toward the *de novo* formation of new hair follicles. However, the procedure takes longer time to perform. It also requires a specialized chamber to fit the wound shape, and the animal has to carry the cumbersome chamber during the wound healing process for a week. Although it is a useful assay for evaluating the efficacy of stem cell candidates to form hairs, it is not practical for a larger-scale screening or future clinical applications. A simplified procedure was developed by injecting much smaller amounts of dissociated precursor cells underneath the skin of mice.⁵ In comparison, this procedure (called the patch assay by its authors) is much easier to perform, and allows for large-scale screening. However, most of the time the patch assay leads to the formation of misaligned hair filaments growing in subcutaneous cysts. While these hair follicles cycle, they cannot cycle normally. Thus, this procedure is useful for evaluating the efficacy of

molecules or candidate cells on hair formation in a short-term basis. However, the procedure cannot become the basis toward practical clinical applications in the future.

Therefore, while useful procedures have been developed and progress has been made,⁶ there is still a need to develop a simple and high-throughput procedure that can generate a large number of pilosebaceous units with a clinically acceptable appearance. Earlier, our laboratory was able to use dissociated feather precursor cells to reconstitute feather follicles *in vitro*. These feather follicles were formed on a plane with proper arrangement and orientation.⁷ This was done *in vitro* by allowing dissociated mesenchymal cells to form a high-density cell suspension. Within a few hours, these cells generated their natural matrix. Epidermis was then laid on top. In this composite, feather progenitor cells self-organize to form periodically arranged feather follicles. Based on this experience, we now devise a new procedure that allows mouse hair precursor cells to generate a large number of hair follicles, which are arranged properly in a plane. These hair follicles can cycle and regenerate, and the reconstituted skin can heal after injury. While this line of research is still a work in progress, this procedure represents a significant step forward toward practical applications in the future.

Materials and Methods

Cell isolation

Multipotential skin precursor cells are currently obtained from neonatal mice using techniques from previously

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published work. Briefly, neonatal mice are harvested shortly after birth (within the first 24 h) and euthanized. The trunk skin is dissected with sharp forceps. Epidermis and dermis are separated by floating the skin in cold 0.25% trypsin solution overnight. Epidermal cells are then dissociated into a cell suspension by cutting into fine pieces and manual titration with a serological pipette. Single epithelial cells are filtered through a 70 μ m cell strainer to exclude cells of the stratum corneum. The dermal cells are individually dissociated using warm 0.35% collagenase solution for 40–50 min at 37°C. DNase I is added for 5 min at room temperature before manual titration with a serological pipette. The collagenase and trypsin activities are stopped by washing cells in either trypsin inhibitor or medium containing a 10% fetal bovine serum. The cells are filtered through a 40 μ m cell strainer to ensure single cell suspension and exclude as many of the preformed hair follicles as possible. Both sets of cells are then recombined in a ratio of 1 epidermal to 5–10 dermal cells and washed again in DMEM:F12 (1:1). The cells are finally resuspended into 150–200 μ L of DMEM:F12 (1:1) as a slurry.

Cell preparation and grafting

Since these are developed in this study, they are presented in the first part of the Results section.

Tissue culture inserts and matrix used

“Integra™ Bilayer Wound Matrix” is used. It is commercially available and is from Integra LifeSciences. This Integra Bilayer Wound Matrix has an integral layer of the semi-permeable silicone membrane that also works as a support for the cell mixture before grafting. For tissue culture insert, it is from BD Falcon. The culture insert membrane is made of polyethylene terephthalate.

Characterization of the reconstituted skin

Skin at different stages were removed. Paraffin sections were prepared. H&E staining and immunostaining were performed as described.⁸ Antibody to neural cell adhesion molecules (NCAM) is described by Chuong *et al.*⁹ Antibody to keratin 14 is from Berkeley Antibody Company. AE13 and Involucrin are from Abcam. Versican is from Millipore.

Wound healing

Mice were anesthetized and small full-thickness wound was produced as described.⁸ These procedures were approved by the USC IACUC.

Hair regeneration after plucking

The regenerative hairs were stripped with warm paraffin or direct extraction using forceps under anesthesia. Pictures were taken every 2–3 days to record hair regeneration.¹⁰

Monitor of hair cycling

This was described in Plikus and Chuong¹¹ and Plikus *et al.*¹² The regenerative hairs were clipped with a human hair trimmer as used in local barbershops. Pictures were taken every 2–3 days to record hair growth. For this purpose, hairs have to be trimmed anew every time immediately before a photo is to be taken.

Results

A simplified procedure to generate hairs arranged in a single normally oriented plane

Preparation of cells for transplantation. Here we use newborn epidermal and dermal cells to generate hairs. The newborn epidermal and dermal cell mixture is known to contain multipotential skin stem cells. Dorsal skins are obtained from newborn mice, and processed into dissociated cells. In average, we can obtain 10^7 epidermal and 10^8 dermal cells from one newborn mouse. After washing, epidermal and dermal cells are recombined in a defined ratio and are resuspended into a very small amount of medium (DMEM/F12 in the ratio of 1/1). This slurry of recombined cells is adjusted to be about 10–100 million cells/mL.

Dissociated epidermal and dermal stem cell candidates are prepared. They can be mixed in different ratios. We then place the cell slurry as a drop with minimal amount of media. Usually, 150–200 μ L of cell suspension, containing 2–20 million cells, was pipetted onto a tissue cell culture insert. At this volume, cells can be held together as a drop by surface tension. If a larger area is desired, cells can also be constrained by a plastic well (we have used those ranging in size from 5–15 mm in diameter). The cell slurries are allowed to settle for 1–2 h in a 37°C incubator, and excess liquid is allowed to evaporate (Fig. 1D). These conditions allow cells to generate a gel-like endogenous matrix. Casting cells into specifically shaped plastic wells allows us to generate skin in freeform.

It is also possible to seed these cells into commercially available matrices. We have tried to seed cells into Matrigel™ or Integra. When Integra is used, the matrix is first rinsed several times with a serum-free medium. An advantage here is that it has a silicone supportive layer. The Integra matrix is cut into the desired size and shape, and blotted dry on sterile, nonstick gauze (with the silicone protective layer on the bottom). The cell slurry is then pipetted evenly onto the undersurface of the dry collagen matrix.¹³ The Integra is about 1 mm thick. For each 1.5 cm² piece of Integra, we typically use ~12 million epidermal cells and 60 million dermal cells in 200 μ L of serum-free medium. Processing is similar for use with Matrigel.

Similar to Zheng *et al.*,⁵ we have worked with different cell ratios, between the epidermal and dermal populations and found that with our technique, a ratio of 1:5–10 for epidermal:dermal cells is optimal.

Grafting to the host. Athymic nude, hairy SCID, or normal mice of the same inbred strain were prepared and draped with betadine solution under anesthesia (Fig. 1A). The intended area of skin to be grafted for hair bearing is excised in full thickness, leaving the musculature beneath undamaged (Fig. 1B, C). Bleeding is controlled with gentle pressure, and the tissue culture insert or collagen matrix, with cells on top or seeded inside, is flipped onto the wound. Cells are pressed against the wound bed with the insert membrane or the silicone protective layer level with the host skin epidermis (Fig. 1E). The membrane is sutured to the host skin (Fig. 1F, G). Sterile dressings are applied to provide constant pressure against the graft to the wound bed (Fig. H–L) so the graft has the best chance of being incorporated to the skin of the host.

FIG. 1. Step-by-step pictorial of the protocol. **(A)** Mice are cleaned and prepared for surgery under anesthesia. **(B, C)** The approximate area of skin to be grafted for hair bearing is cut out in a full-thickness layer, note musculature. **(D)** An example of scaffold that has been seeded with cells using a pipette and allowed to dry briefly. Scaffold is sitting on a protective silicone membrane. **(E)** Placement of scaffold and cells over recipient bed. **(F, G)** Simple interrupted sutures to secure graft in place. **(H, I)** Antimicrobial ointment and gauze used to dress the wound. **(J-L)** Securing of dressing with a tight elastic wrapping allows for better adherence to wound. The mice have no restrictions during the postoperative period. Dressings are removed 7–12 days later. The silicone protective layer is easily peeled off once the wound has re-epithelialized. Color images available online at www.liebertonline.com/tec.

Dressings are removed for inspection around days 8 postgrafting. The sutures are removed and the protective silicone layer or insert can now be peeled off easily because the wounds have been re-epithelialized. Once dressings are removed, no special care of the animal is needed.

Characterization of reconstituted hairs

Hairs can be seen by the naked eye on the surface of the wound as early as 11–15 days postgraft. We have high reproducibility of hair formation (Fig. 2B). Here we describe their arrangement in the gross view and molecular characterization on histological sections. The hair forms densely on the reconstituted skin and hairs are arranged on a plane and grow evenly in a cosmetically acceptable fashion (Fig. 2A–D). Under a microscope, hair filament shows a normal appearance. Differences are not apparent when the assay is performed with a tissue culture insert or with a commercial matrix. Sometimes there is higher hair density

closer to the wound margin, and tissue sections suggest that it is due to the accumulation of more cells around the sutures.

Controls include the use of Integra or Matrigel without cells. In these cases skin healed by wound contraction and re-epithelialization without new hair formation.

Histological sections of the skin at day 11 postgraft show that normal layers of the skin, including the epidermis, hair follicles, sebaceous glands, subcutaneous adipose layer, and dermis, have been recreated (Fig. 3). They are arranged with the right architecture and each component is of the right size and shape. The epidermis is somewhat wrinkled at this stage, which becomes flat later (Fig. 7A). We used several immunohistochemical molecular markers to monitor their molecular differentiation (Fig. 3). K14 is present in the basal layer and the follicle sheath. NCAM is mainly present in the dermal papilla. Versican is present strongly in the dermal papilla. Involucrin is present in the suprabasal keratinocytes. AE13 is expressed in the inner root sheath. Oil red O is

FIG. 2. Generation of planar arranged hairs. **(A)** Macroscopic evidence of hair growth as soon as dressings are removed on day 11. **(B, C)** Full growth of hair over grafted region by day 21. **(D)** Close up view of the hairs. **(E)** The number of experiments and outcome. +++, the transplanted area is all covered by hairs; ++, >75% of the transplanted area is covered by hairs; +, >50% of the transplanted area is covered by hairs. Color images available online at www.liebertonline.com/tec.

positive in the sebaceous gland and the subcutaneous adipose tissue. These staining patterns are similar to those reported for normal hair follicles.

Morphogenetic process of reconstituted skin

To study the morphogenetic process that takes place in the formation of new hair follicles, we prepare sections using

specimens obtained from different postoperative days. We follow-up the appearance of molecular markers from day 4 to 9 postgraft (Fig. 4).

K14. At day 4 postgraft, K14-positive cells scatter around, without forming a sheet or aggregates, in the matrix. At day 5 postgraft, the K14-positive cells start to coalesce and organize themselves into a basal epidermal layer. At day 8, hair pegs can be seen to invaginating into the newly generated dermis.

NCAM. NCAM-positive cells can be seen at day 4, distributed randomly in the matrix. At days 5 and 7, they can be seen to be distributed in the dermis, and become enriched in the dermal papilla at days 8 and 9 when the morphology of hair follicles become clear.

Involucrin. Positive cells appear at day 5 in the putative epidermis. At day 7, it is expressed in the suprabasal cells facing the cavity flanked by the invaginated epidermis. At day 9, it is limited to the suprabasal epidermis facing the outside.

Versican. Expression is similar to NCAM. Positive cells are first distributed in the dermis randomly at day 4, and eventually and by day 8 have homed in to the dermal papilla.

Putting these together, we began by mixing epidermal and dermal cells randomly together. At day 4, these cells still appear randomly mixed, but cell re-arrangement has started to take place in this 1-mm-thick matrix. At day 5, epidermal cells sort themselves out and coalesce to form a basal layer first at the bottom of the matrix. They then rise from the base to the level of the air surface level (Fig. 4). Some transient epidermal microcysts can be observed at days 7 and 8. Dermal cells started to form periodically arranged dermal condensations adjacent to the epidermal layer or cyst. The epidermis eventually rises to the surface and flattens out. At day 8, hair germs start to appear, which progress to the hair peg stage at about day 9. Dermal condensations also progress to form dermal papillae. Together, they form hair follicles at about days 11–12.

The orientations of these hair follicles are all pointing toward the epidermal surface. The formed hair follicles point upward and hair filaments are able to protrude out to the surface of the skin. We have never observed hair follicles pointing to the underside of the matrix or horizontally. When the matrix is overloaded with too many cells, occasionally some cysts remain in the dermis that fail to merge with epidermis. Some hairs can grow into these cavities, similar to those observed in the patch assay.⁵

Reconstituted hairs cycle physiologically and can regenerate after plucking

One of the criteria to judge successful formation of engineered hair follicles is the ability of the follicle to cycle physiologically and to regenerate after plucking.¹⁴ Here, we examined their physiological cycling. Hairs were clipped short to allow hair cycle phases to be observed. Indeed, we can see that after hairs were clipped they can still grow and achieve normal length in 2 months (Fig. 5A). The hairs can cycle continuously up to more than 1 year.

We then tested their regenerative ability after hairs are plucked. We initially tested small patches of hair directly

FIG. 3. Molecular characterization of the reconstituted skin. Sections of the reconstituted skin and immunostaining of molecular markers. Involucrin is in the epidermis. NCAM is mainly in the dermal papilla. Oil red O is shown in the sebaceous glands and subcutaneous adipose tissue. AE13 stains positive in the inner root sheath. K14 is in the epidermis and outer root sheath. Versican is in the dermal papilla. NCAM, neural cell adhesion molecules. Color images available online at www.liebertonline.com/tec.

FIG. 4. Cellular and molecular events during the process of hair reconstitution. H&E staining reveals that cells start at the base of the scaffold near the wound bed and migrate to the surface as the cells differentiate and organize themselves into pilosebaceous units within normal skin. K14: There is evidence of basal keratinocytes scattered throughout the matrix initially. They then organize themselves into a basal epidermal layer. NCAM: Positive cells organize themselves over the course of time to the subepidermal layer. Involucrin: Positive cells organize themselves into the basal epidermal layer reconstituting normal epidermis and hair shaft. Versican: Positive cells begin in the same layer as all other cells and by day 8 have homed to the dermal papilla. Note that hair follicle orientation is then readjusted toward the epidermal interface. Color images available online at www.liebertonline.com/tec.

plucked individually by forceps. Once we found that plucked hairs could regenerate, we advanced to larger areas of hair removal to look at patterns of anagen. Hairs in the whole graft were plucked with warm wax. These hair follicles re-enter anagen in about 10 days, and pigmented anagen follicles are visible in 2 weeks. They continued to grow and reach the normal length in about 2 months (Fig. 5B).

Reconstituted skin lasts more than 1 year and can respond to injury and heal

We wondered about the stability of the reconstituted skin and how they respond to a full-thickness wound. We tested

this by making a 3-mm full-thickness punch wound on the reconstituted skin (Fig. 6, arrow). When 3-mm full-thickness wound was made in normal mice, it takes 14 days to close. When the same kind of wound was made on the reconstituted skin, it also takes 14 days to close (Fig. 6B). Hairs around the wound margin now grow faster, similar to the report that a wound itself can stimulate the growth of existing hair follicles.

In terms of long-term stability, we have produced reconstituted skin and are able to keep them up to 18 months. The hairs are still growing and cycling. Sections show reconstituted skin can form apparently normal epidermis and dermis that integrates with the host skin (Fig. 7). Hair follicles

FIG. 5. Reconstituted hairs can regenerate. **(A)** Physiological hair cycling. Skin is 6 months after planar hair transplantation. At time 0, hairs were clipped with a trimmer. At 2–4 weeks later, hair was again clipped and photographed. The pigmented area represents anagen; the pink area represents telogen. Note that pigmented regions in the 2-week picture have become pink by 3 weeks. Similarly pigmented regions change in the 4-week photo, implying changes associated with hair cycling. **(B)** Hair regeneration after plucking. Mouse is 1 year after planar hair transplantation. The reconstituted skin was stripped with warm wax, which is similar to plucking over a large area. After hair plucking, the region is pinkish (time 0). At 2 weeks, the region shows pigmentation, implying follicles below have entered anagen. At 3 and 4 weeks after plucking, hair continues to grow. Color images available online at www.liebertonline.com/tec.

FIG. 6. Reconstituted skin is able to respond to full-thickness wounding and heal. **(A)** Approximately 3-mm full-thickness wounds were produced (blue arrow). Three days after wounding the opened wound can be seen. Five days after wounding, the wounds became smaller. Ten days after wounding, the wound is closed. Note that the hairs to the left of the wound seem to grow robustly. **(B)** Diagram showing the rate of wound closure. pww, days postwounding. Color images available online at www.liebertonline.com/tec.

and subcutaneous adipose tissue are also observed. However, the subcutaneous muscle layer is not reformed. There are no differences of skin quality or hair growth when tissue culture insert, Matrigel, or Integra are used.

Potential future applications of this procedure

Since one of the major motivations of this work is to screen for candidate genes that can reprogram adult epidermal or dermal cells into multipotential skin cells, we tested if lentivirus transduced cells can form hairs normally. Lentivirus carrying green fluorescent protein (GFP) was used to transduce newborn skin cells, either dermal or epidermal. As seen by GFP, the transduction efficiency was high and these cells also moved on to form hairs normally (Fig. 8A). So, they can be used to test if the lentivirus-mediated suppression or upregulation of certain genes are essential for new hair formation.

For future clinical use, we need to be able to accommodate irregular sizes and shapes of the wounds. Since the matrix, whether endogenously produced or exogenous, is reasonably stiff, it can be shaped to desired forms (Fig. 8B–D). This can conceivably be made clinically useful in the reconstructive procedures of hair replacement of particularly shaped regions of hair growth (i.e., eyebrows). In terms of the size of the reconstituted skin, we have made grafts of about 500 mm² surface area on the mouse with successful hair growth. This is ~30% of a mouse's total body surface area. Additionally, a few of our mice have endured multiple grafts

in different parts of their bodies. This procedure is also tolerable in multiple stages.

For the potential use toward alopecia, we may want to make the region as small as possible. We have made grafts as small as 0.5 cm² with successful hair formation. Thus, the reconstituted skin can be made with flexibility in shape and size.

Discussion

In the age of tissue engineering, there is desire to reconstitute hair follicles from dissociated single cells.¹⁵ The cells have to be multipotential stem cells. The challenge is that these cells not only have to differentiate into different cell types, they have to be arranged with the proper organization. This is important at the level of intrafollicular organization, as well as in the arrangement of a population of follicles. Here we describe an improved and simplified procedure that allows multipotential skin precursor cells to form a large number of *de novo* hair follicles on a plane. These cells self-organize in a plane, forming a skin with a cosmetically acceptable appearance. Histologically, the reconstituted skin shows proper proportions and topological arrangements of different skin components, including hairs, sebaceous glands, dermis, and subcutaneous adipose tissues, but not the muscle layer.

This work builds on earlier achievements by Lichti^{1,4} and Stenn's group.⁵ Other investigators also have worked to produce high-throughput assays for hair-forming ability.

FIG. 7. Long-term survival of reconstituted skin. After planar hair transplantation, the wound area is recovered by regenerative skin. The skin was traced 4, 9, and 12 months after the procedure. Left column shows gross view. The 9-month specimen has two small and one big graft. Middle column shows skin sections including the wound margin (marked by arrow). In these specimens, hair follicles are in telogen stage. The right column shows reconstituted skin with anagen hair follicles. The skin shows normal architecture with subcutaneous adipose tissue, but not muscle. Duration after the graft and procedure used are indicated. Color images available online at www.liebertonline.com/tec.

Matrix and insert membrane 4 month after transplant

Insert membrane 9 month after transplant

Integra 1 year after transplant

Havlickova *et al.*¹⁶ have developed a way to evaluate the effect of different molecules on hair formation. However, the formed structures do not progress into real hair follicles. Different methods to manipulate the matrix and dermal cell aggregates have been developed to facilitate the formation of

reconstituted skin and development of hairs with different levels of success.^{17–20} Our procedure is advantageous in that it can be performed efficiently and on a large scale so that it can be used for a high-throughput screening of molecules important for the formation of hair follicles.

FIG. 8. Future potential of reconstituted skin. (A) Lentivirus was used to transduce cells and hairs can still form. Green fluorescent protein (GFP) indicates cells transduced with lentivirus containing GFP. The hairs form normally. (B–D) Flexibility in shaping of the reconstituted skin. Using a reasonably stiff matrix to hold multipotential cells, the graft can be cut to specific shapes and sizes for cosmetic applications. Color images available online at www.liebertonline.com/tec.

It is critical that the engineered hair follicles fulfill the definition of hair follicles. Because of this, we have earlier developed a definition of hair follicles that includes the concentric hair filament organization, proper hair follicle configuration, stem cell and transient amplifying cell topology that allows proximal-distal growth, the association of sebaceous glands, and the ability to save stem cells for repetitive cycling.¹⁴ Here we evaluate our reconstituted skin hair follicles with these criteria. We show that the reconstituted hair follicles show the proper follicular organization, express hair differentiation markers, and contain sebaceous glands. The reconstituted hair cycles under physiological conditions. They also respond to injuries caused by hair plucking and wax stripping and regenerate properly. Further, we have injured the skin with full-thickness wounds. Skin healing can take place properly in a timely fashion. We also followed up the reconstituted skin on the mice that has been transplanted up to 18 months. Hair growth and cycling are still active. Thus, with multipotential skin stem cells, this procedure provides an excellent platform for the formation of reconstituted skin.

While the hair-forming process parallels that in development, by analyzing the morphogenetic process in regeneration, we observed several novel morphogenetic processes that do not occur in development. (1) The originally randomly distributed epidermal and dermal cells gradually sort themselves out. This may be explained by the more adhesive force among epidermal cells compared to the epidermal-dermal adhesiveness, or the dermal-dermal adhesiveness.²⁰ (2) At day 5 after grafting, most epidermal cells are near the bottom of the graft. The second surprise is that these epidermal cells, in the form of aggregates or dissociated cells, start to shift upward toward the air surface. During this process, some small epidermal cellular aggregates are seen in the process of coalescing as they rise. They become connected to the epidermis in the wound margin of the host skin and flatten out. Eventually, a flat epidermal layer lies on top of the dermal layer. (3) The ability of dermal cells to form periodically arranged dermal condensations adjacent to the epidermal cells. These dermal condensations and the adjacent epidermis then progress to become follicles (Fig. 9).

How these different processes occur at the cellular and molecular level constitute future challenge. For example, the mechanism for epidermal cells to form aggregates/cysts or flat configurations is most interesting. It probably involves changes of cell adhesion and mechanics. The mechanism by which this occurs and the establishment of correct apical-basal cellular polarity pose other mysteries. We speculate that epidermal cells may be attracted by an oxygen gradient. For periodic patterning, we think the mouse dermal and epidermal cells we deliver at this stage are still competent for morphogenesis, and can respond to the activators and inhibitors, leading to the periodic pattern formation of skin appendages.²¹ This process provides the possibility to modulate the size of hair germs by altering the concentration of these activator and inhibitor activities.⁷ Thus, this model provides us with a great opportunity to study the morphogenetic ability of stem cells during regeneration and reconstitution. Learning to trigger these processes will be key to the success of stem cell engineering in the future.

Molecular re-programming has now opened up possibilities to switch the fate of cells.²² A similar strategy can be

FIG. 9. Schematic drawing of the hair reconstitution process in this procedure. Green, epidermal cells; red, dermal cells. Cells are mixed randomly in the three dimensional matrix. Epidermal cells begin to sort themselves out and coalesce to form a layer first near the bottom of the matrix. They then “rise” from the base to the level of the air surface level. Some transient microepidermal “cysts” can be observed. Dermal cells also start to form condensations adjacent to the “rising” epidermis. The epidermis eventually flattened out at the surface. Hair germs appear periodically. They progress to form hair pegs, and then onto form hair follicles. The morphogenetic process occurs between about day 5 to 12 after grafting. Color images available online at www.liebertonline.com/tec.

used to reprogram somatic cells to gain or lose the ability to form hairs. To achieve this potential in drug discovery, we need a simplified procedure to screen for small molecules or genes with a clear readout. This procedure provides an efficient large-scale screening of small molecules. To pave the way for future molecular screening, we transduced these cells with lentivirus carrying GFP, and show hairs can still form normally. Thus, we can use lentivirus to overexpress or suppress candidate genes that are involved in hair growth.

In summary, this is a simple one-step procedure in which cells are able to self-organize and differentiate properly to generate reconstituted skins. A large number of pilosebaceous units are generated and distributed in a plane with a cosmetically acceptable arrangement. The graft can be made with flexibility into flexible shape and size. Thus, while this procedure is a work in progress, it is a model for translational research and clinical applications can be envisioned for the future when large numbers of multipotential skin stem cells become available.

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Disclosure Statement

Provisional patent "Compositions and Methods to Generate Pilosebaceous Units U.S. Patent application No. 61/116,620" was filed by University of Southern California on November 20, 2008.

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