# *De Novo* Epidermal Regeneration Using Human Eccrine Sweat Gland Cells: Higher Competence of Secretory over Absorptive Cells

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In our previous work, we showed that human sweat gland-derived epithelial cells represent an alternative source of keratinocytes to grow a near normal autologous epidermis. The role of subtypes of sweat gland cells in epidermal regeneration and maintenance remained unclear. In this study, we compare the regenerative potential of both secretory and absorptive sweat gland cell subpopulations. We demonstrate the superiority of secretory over absorptive cells in forming a new epidermis on two levels: first, the proliferative and colony-forming efficiencies *in vitro* are significantly higher for secretory cells (SCs), and second, SCs show a higher frequency of successful epidermis formation as well as an increase in the thickness of the formed epidermis in the *in vitro* and *in vivo* functional analyses using a 3D dermo-epidermal skin model. However, the ability of forming functional skin substitutes is not limited to SCs, which supports the hypothesis that multiple subtypes of sweat gland epithelial cells hold regenerative properties, while the existence and exact localization of a keratinocyte stem cell population in the human eccrine sweat gland remain elusive.

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## INTRODUCTION

Human epidermal homeostatic renewal and re-epithelialization after injury are promoted by keratinocyte stem cells, which are thought to reside in the basal layer of the epidermis (Strachan and Ghadially, 2008) and in skin appendages (Brouard and Barrandon, 2003; Blanpain and Fuchs, 2006). In the latter, eccrine sweat glands (ECGs) seem to act as major contributors to wound re-epithelialization (Rittie *et al.*, 2013). However, the only multipotent stem cell population of the skin characterized so far is located in the bulge region of the hair follicle (reviewed in Alonso and Fuchs, 2003; Cotsarelis, 2006). Sebocytes appear to be an early direct product of bulge cells (Frances and Niemann, 2012). Outer root sheath cells of hair follicles, originating from the bulge, differentiate *in vitro* into a fully stratified epidermis (Lenoir *et al.*, 1988; Limat *et al.*, 2003), and bulge stem cells contribute in mice to epidermal regeneration after wounding, yet not to homeostatic epidermal renewal (Ito *et al.*, 2005; Levy *et al.*, 2007). In contrast, sweat glands of mouse paw pads show a substantial turnover during both wound repair and homeostasis (Lu *et al.*, 2012).

In our previous work, we demonstrated that human ESG cells have the ability to reconstitute ''*de novo*'' a correctly stratified, interfollicular epidermis with a pronounced stratum corneum and a functional basal layer, which is firmly anchored to the basement membrane. The epidermal marker expression pattern was nearly identical to that of an epidermis derived from bona fide keratinocytes (Biedermann *et al.*, 2010). Additionally, sweat gland–derived epithelial cells also showed the ability to incorporate melanin, an important property of epidermal keratinocytes (Böttcher-Haberzeth *et al.*, 2012). Therefore, we concluded that sweat gland–derived epithelial cells represent a source of keratinocytes capable of growing a near normal autologous epidermis.

Anatomically, the ESG is a tubular structure, originating in the epidermis and blindly ending in the reticular dermis. It basically consists of two main segments: the distal absorptive and the proximal secretory duct. The absorptive duct is a stratified bi-layer composed of a peripheral and an inner luminal layer of absorptive cells (ACs). It can be subdivided into acrosyringium (intra-epidermal duct), straight (intradermal) duct, and intraglandular coiled duct (Figure 1a). The secretory duct consists of a pseudostratified single cell layer, which is composed of alternating clear, secretory, and dark, mucoid cells and is surrounded by myoepithelial cells (Langbein *et al.*, 2005). An interesting question raised by our previous work (Biedermann *et al.*, 2010) is: which subtype of sweat gland cells is involved in epidermal regeneration and

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*Abbreviations: AC, absorptive cell; DESS, dermo-epidermal skin substitute; ESG, eccrine sweat gland; SC, secretory cell*

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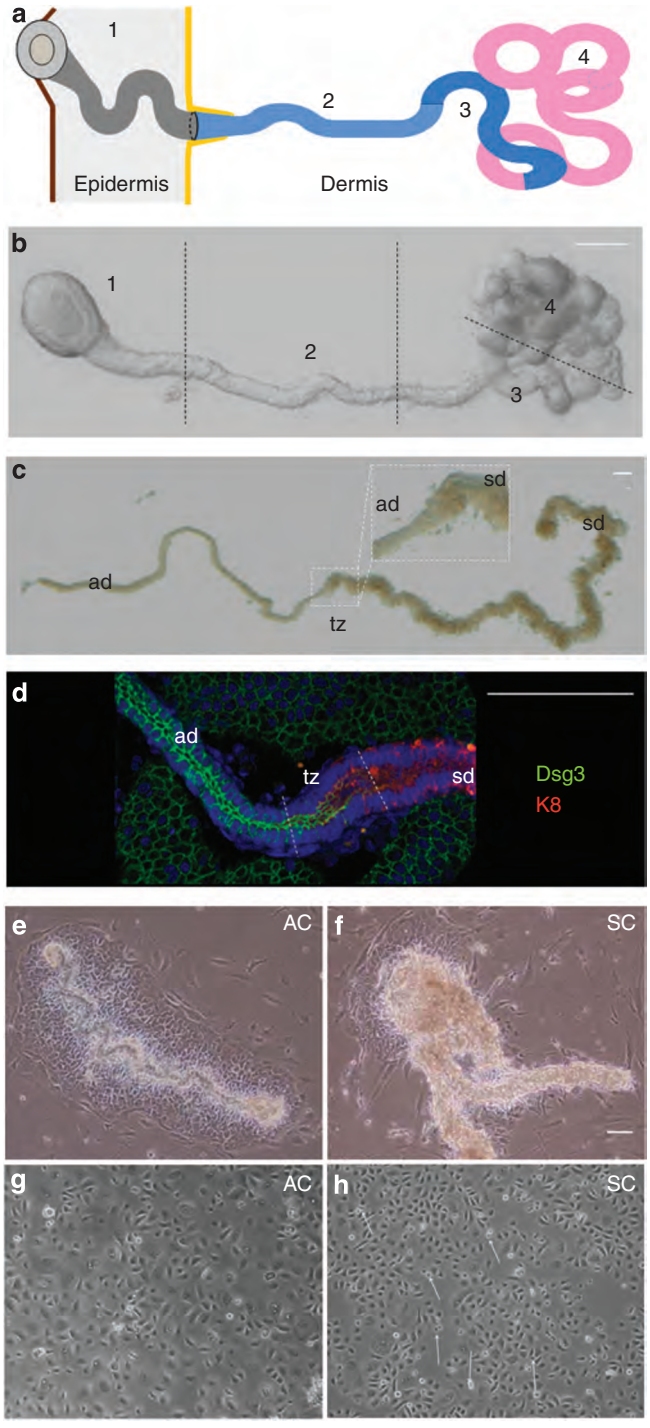


**Figure 1. Isolation of pure populations of absorptive (ACs) and secretory cells (SCs) from the human eccrine sweat gland (ESG).** (**a**) Illustration of the human ESG emphasizing four domains: Acrosyringium (intra-epidermal duct) (1), straight (2), and coiled (3) absorptive and secretory ducts (4). (**b**) Isolated ESG showing the same structural regions. (**c**) Streched gland showing absorptive (ad) and secretory ducts (sd) by light stereomicroscopy. The box illustrates a 2.6-fold magnification of the transition zone (tz) between the two main regions. (**d**) Whole-mount immunofluorescence staining of ESG. The luminal cells of the absortpive duct express desmoglein 3 (Dsg3; green), and the SCs express keratin 8 (K8; red). (**e**, **f**) Absorptive and secretory ducts cultivated on feeder cells for 6 days (note the outgrowing cells) and (**g**, **h**) after three passages in culture. The white arrows in **h** indicate some cell divisions. Bars = 100 μm.

maintenance? *In vivo* proliferative activity is found mainly in the absorptive domain of sweat glands *in vivo* (Langbein *et al.*, 2005; Li *et al.*, 2008; Lu *et al.*, 2012). However, the presence of a label-retaining (stem) cell (LRC) population is reported (in humans and mice) exclusively in the secretory domain (Nakamura and Tokura, 2009; Lu *et al.*, 2012).

The objective of our current study was to determine the role of human ESG cell subpopulations in skin regeneration and homeostasis and to test the hypothesis that the secretory domain of the human ESG may harbor an epidermal stem cell niche. To achieve this, we separately isolated cells from the absorptive duct (ACs) and from the secretory portion (secretory cells (SCs)) of the human ESG. We included ACs and SCs in organotypic cultures consisting of a stratified dermo-epidermal skin substitute (DESS). We show that cells gained from the secretory duct of the ESG are endowed with a clearly higher viability in culture and allowed to obtain high quality DESS *in vivo* and *in vitro* in a more reproducible way than ACs.

## RESULTS

### Isolation and discrimination of the absorptive and secretory domains of the ESG

We isolated human ESG (Figure 1b) from different body regions and from donors of different age groups (Supplementary Table S1 online). The transition zone between the dermal duct and secretory coil was clearly distinguishable using a stereo microscope (Figure 1c, tz). In addition to the visual discrimination, the two different gland domains were identified by whole-mount immunofluorescence using antibodies against desmoglein-3 (Dsg3), expressed exclusively in the absorptive duct (Figure 1d, ad), and keratin 8 (K8), limited to the secretory duct (Figure 1d, sd). To avoid possible contaminations and to obtain pure ACs and SCs, the short transition zone fragment was excised and eliminated. Absorptive and secretory fragments were cultivated separately on feeder cells until the outgrowing cells (Figure 1e and f and Supplementary Figure S7c online) had formed consistent colonies. The cells were passaged and expanded on collagen I–coated dishes in a serum-free, low calcium medium. The differences in size and morphology became evident after 2–3 passages: the AC population (Figure 1g, Supplementary Figure S2a, S2c, and S2e online) appeared inhomogeneous with rare mitotic and numerous big, terminally differentiated cells, whereas SCs (Figure 1h, Supplementary Figure S2b, S2d, and S2f online) were smaller in size, had a homogeneous cobblestone morphology, and revealed the presence of numerous small cell pairs as an immediate product of cell division (Figure 1h, white arrows).

### Absorptive and secretory ducts express different markers, whose expression pattern is modified in culture

Many proteins are expressed specifically in SCs or ACs in normal skin (Supplementary Figure S1a online). We performed repeated analyses of the RNA and protein expression pattern by PCR and immunofluorescence to characterize the nature of the obtained cell cultures. We found that the original expression pattern of several markers was not maintained in culture.