Generation of *in vitro* skin models using hair follicle-derived cells

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Introduction

Due to growing social and political pressure, the interest in alternatives to animal testing has constantly increased during the past 10 years stimulating the development and validation of new *in vitro* test systems including reconstructed skin models. Additional to toxicological studies and permeability assays, skin models are of high interest for fundamental research to elucidate basic physiological and pathophysiological processes in human skin [1, 2]. As for today, most of the *in vitro* skin models are grown from primary keratinocytes and fibroblasts that were either isolated from excised human skin or from juvenile foreskin following circumcision. In contrast plucked hair follicle has become of more interest for generating epidermal equivalent not least because of the less-invasive access.

Aim

- **Generation of an *in vitro* full-thickness skin models using hair follicle-derived cells**
- **Investigation of different methods to optimize keratinocytes and fibroblasts isolation and expansion of hair follicle-derived keratinocytes (HFDK) as well as hair follicle-derived fibroblasts (HFDF)**
- **Characterization of hair follicle-derived cells**
- **Comparison of full-thickness skin models grown from skin-derived cells and hair follicle-derived cells**

Methods

Isolation of hair follicle-derived cells

Hair follicles were plucked from the scalp of volunteers and those in the anagen phase were selected and rinsed four times in washing medium containing antibiotics. Afterwards, the cells were isolated using different methods including direct trypsinization or outgrowth of the cells by co-culture with postmitotic normal human dermal fibroblast (NHDF) [3]. To prevent the floating of the hair follicle on liquid surface a nylon mash or the cell culture inserts Transwell 3450 (Corning Costar, Cambridge, USA) or BD 353090 (BD Biosciences, Franklin Lakes, USA) was used. After cultivation for 14 days, the cells were trypsinized and the cell yield was determined by cell counting.

Secondary cultivation of hair follicle-derived cells

Following isolation, the cells were either further cultivated with feeder cells in specific serum-enriched cell culture medium to expand HFDK or using the same culture medium without feeder cells to obtain HFDF. After 5 days, HFDF were further cultured in DMEM supplemented with 10 % fetal calf serum and L-glutamine.

Characterization of hair follicle-derived cells

The hair follicle-derived cells were characterized on protein level by immunostaining for the fibroblast-specific markers vimentin (VIM) and desmin (DES) and the keratinocyte marker cytokeratin (CK) 5, 10, 14 and 17. Moreover, the relative mRNA expression of type I collagen (Col I) and type IV (Col IV), TGF-beta, alpha smooth muscle actin (SMA), IL-1 alpha, IL-6 and IL-8 was quantified via quantitative PCR.

Generation and characterization of skin models

Skin models were generated according to previously published procedures [2]. Normal human epidermal keratinocytes (NHEK) and normal human dermal fibroblasts (NHDF) were isolated from juvenile foreskin. Cells were used in passage 4-5. After 14 days, the skin models were analyzed using H&E staining and immunostaining for CK14, Involucrin (IVL) and Filaggrin (FLG).

Results

Direct outgrowth of hair follicle-derived cells on cell culture insert

The best procedure for isolation of hair follicle-derived cells with the highest cell yield was the direct outgrowth on Transwell 3450 by co-culture with postmitotic NHDF on the basal side (Fig. 1) [4].

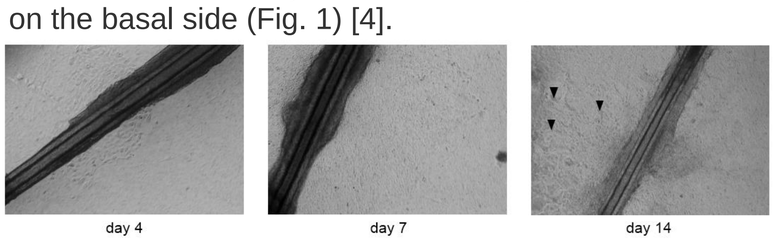

Figure 1 | Outgrowth of hair follicle cells on Transwell 3450 by co-culture with a feeder layer on the basal side for a period of 14 days. Magnification 10 x.

Characterization of hair follicle-derived cells

Following cultivation of hair follicle-derived cells in serum-enriched medium the cells changed their morphology and were growing in fibroblast-specific wave-like pattern after a period of 14 days (Fig. 2).

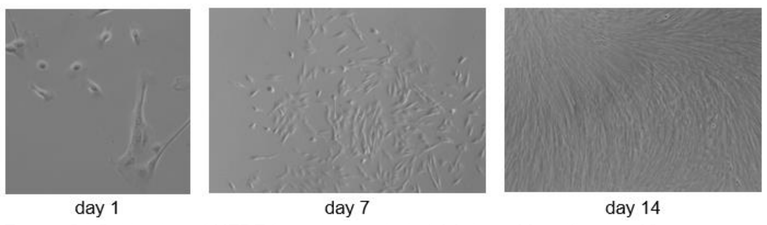

Figure 2 | Cultivation of HFDF for a time period of 14 days. Magnification 10 x.

In the following step, the hair follicle-derived cells were characterized using immunostaining and quantitative PCR for different markers. As control, immunostaining was also performed with NHDF. HFDF as well as NHDF clearly expressed VIM and DES, but were negative for all cytokeratins tested. As expected, HFDK were positive for CK14 and negative for the fibroblast-specific markers (Fig. 3). Moreover, under cultivation conditions HFDK were negative for CK5, a follicular keratinocytes marker, and for CK10, a differentiation marker. However, HFDK expressed CK14, a marker for basal keratinocytes and CK17, a marker for interfollicular keratinocytes (data not shown).

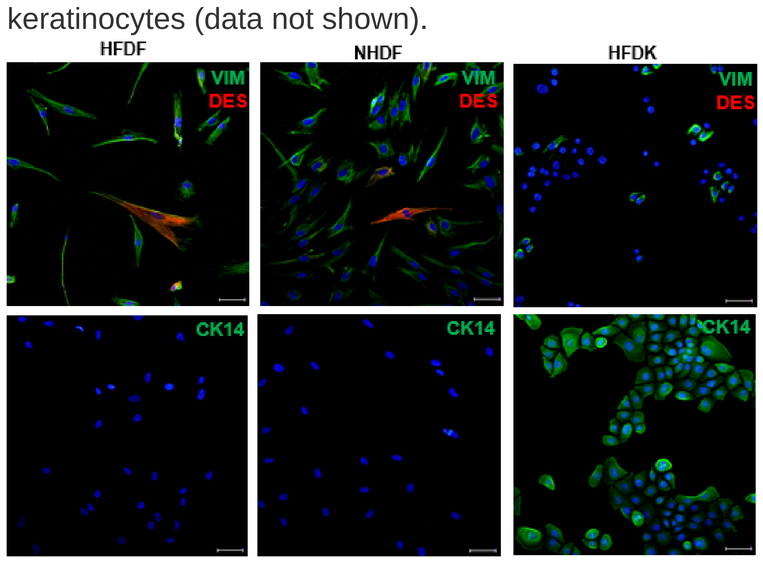

Figure 3 | Immunostaining for VIM, DES and CK14 of HFDF, NHDF and HFDK. Nucleic were stained with DAPI (blue fluorescence). Scale bar = 50 µm.

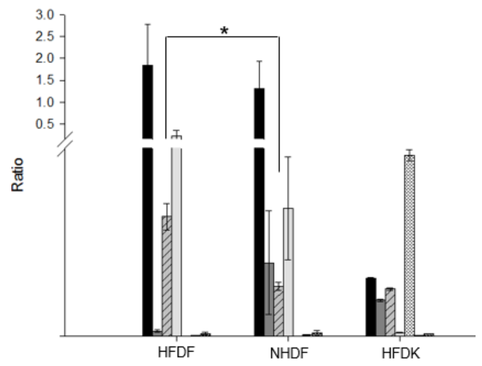

Figure 4 | Relative mRNA expression of different markers in HFDF, NHDF and HFDK. Relative mRNA expression were calculated via target/reference ratio. Mean ± SEM, n = 3.

The expression of Col I, Col IV, TGF-beta, IL-alpha, IL-6 and IL-8 in HFDF and NHDF fibroblasts showed no significant differences (Fig. 4).

The higher expression of alpha SMA in HFDF indicate that the cells may originate from the dermal papilla.

Generation of full-thickness skin models

Skin models grown from skin-derived cells exhibited all epidermal layers also present in native human skin. The same was observed for skin models grown from the hair follicle-derived cells. No differences in histology and differentiation were detected (Fig. 5).

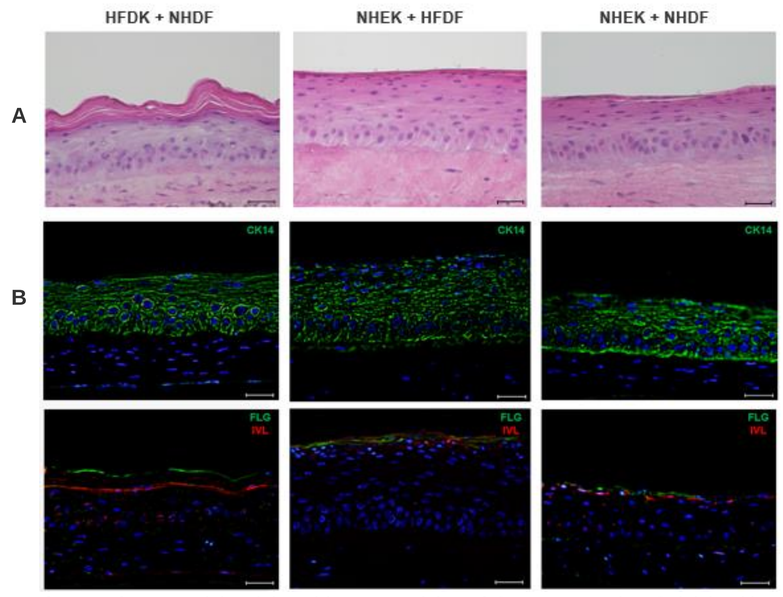

Figure 5 | H&E staining (A) and immunostaining of CK14, IVL and FLG (B) in skin models with hair follicle-derived cells and cells isolated from juvenile foreskin. Scale bar = 100 µm. Nucleic were stained with DAPI (blue fluorescence).

Conclusion

In this study, we compared different **methods for the isolation of hair follicle-derived cells from plucked hairs.** The best procedure with the highest cell yield was **direct outgrowth** of the cells on cell culture inserts (Transwell 3450) by co-culture with a feeder layer on the basal side. Besides, we were able to **isolate, generate and expand keratinocytes and fibroblasts from plucked hair follicles** which did not differ from NHK or NHDF. Moreover, generation of skin models using hair follicle-derived keratinocytes and fibroblasts was performed successfully showing **no differences in epidermal maturation or differentiation from skin models** grown from skin-derived cells.

References

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