Post-Mitotic Human Dermal Fibroblasts Efficiently Support the Growth of Human Follicular Keratinocytes

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For growth at low seeding densities, keratinocytes isolated from human tissues like epidermis or hair follicles are dependent on mesenchyme-derived feeder cells such as the 3T3-cell employed so far. As an alternative method, the present study describes the use of post-mitotic human dermal fibroblasts sublethally irradiated or mitomycin C-treated. Special emphasis was put on efficient growth of primary keratinocyte cultures plated at very low seeding densities. Thus, outer root sheath cells isolated from two anagen human hair follicles and plated in a 35-mm culture dish ($3-6 \times 10^2$ attached cells) grew to confluence within 3 weeks ($6-8 \times 10^5$ cells). Similar results were obtained for interfollicular keratinocytes. A crucial point for the function of these fibroblast feeder cells is plating at appropriate densities, considering their tremendous increase in cell size at the post-mitotic state. Plating densities of $4-5 \times 10^3/cm^2$ allow full spreading of the feeder cells and do not impede the settling and expansion of the keratinocytes. Major advantages of this system include easier handling and better reproducibility than using 3T3-cells. Moreover, homologous fibroblast feeders mimic more closely the physiologic situation and therefore might provide a valuable tool for studying interactions between human mesenchymal and epithelial cells. Finally, potential hazards of using transformed feeder cells from a different species in keratinocyte cultures raised for wound covering in humans could be thus avoided. *J Invest Dermatol 92:758–762, 1989*

In the past, various methods have been developed for the cultivation of keratinocytes, usually disaggregated by combined mechanical and enzymatic action (for review see Ref 1). Thus, human epidermal keratinocytes isolated from keratome sections of adult skin grew well up to at least three passages when plated on a collagen surface [2]. Other approaches included the growth of human epidermal keratinocytes on fibronectin-coated dishes [3] or at reduced Ca^{++}-levels in media containing chemically defined supplements and bovine pituitary extract [4]. This way, large quantities of highly proliferative basal-cell-like keratinocytes have been obtained from small skin biopsies [5].

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Abbreviations:
cGy: Centi-gray(s)
CPD: Cumulative population doublings
DMEM: Dulbecco's modified Eagle's medium
EDTA: Ethylenediaminetetraacetic acid
EGF: Epidermal growth factor
FCS: Fetal calf serum
HDF: Human dermal fibroblasts
HEPES: N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HHF: Human hair follicle(s)
kDa: Kilodalton(s)
NEPHG: Non-equilibrium pH gradient gel
ORS: Outer root sheath(s)
PBS: Phosphate-buffered saline
SDS: Sodium dodecyl sulfate
TGF-α: Transforming growth factor-α

Alternatively, using growth-arrested murine 3T3-cells, Rheinwald and Green reported rapid growth of human neonatal and adult keratinocytes in primary cultures seeded at low density [6]. In fact, the development of this technique had a great impact on biochemical as well as molecular biologic studies and is presently evaluated for the covering of skin defects such as burn wounds [7,8].

In the skin, an additional population of living epithelial cells is located in the outer root sheath (ORS) of hair follicles. Although a series of sophisticated experiments performed by Oliver [9–11] strongly suggests that ORS-cells play some role in the hair-forming process, their exact function during the different phases of the hair cycle still remains to be elucidated. The reepithelialization of superficial skin wounds, on the other hand, represents another essential property of ORS-cells [12], indicating their inherent potential for alternative routes of differentiation. To further investigate the biology of ORS-cells, methods for their cultivation have been developed, mainly by explanting plucked human hair follicles (HHF) on various growth-substrata such as bovine eye lens capsules [13], collagen-coated dishes [14], and tissue-culture plastic [15]. Recently, we reported that primary cultures of dispersed ORS-cells require feeder cells [16] because of the small number of ORS-cells available per plucked HHF and a greatly reduced plating efficiency of this cell type when cultured without a feeder layer (unpublished data). However, frequent problems with the conventional 3T3-feeder technique (ATCC: CCL 92), i.e., poor reproducibility mainly due to degeneration and detachment after growth-arrest, prompted us to look for an alternative feeder system.

In vitro, human dermal fibroblasts (HDF) have recently been demonstrated to represent a population of cells at various differentiating states with distinct morphologic and biochemical characteristics [17–19]. Three mitotic and four post-mitotic types were described, the latter ones displaying a significantly enlarged cell size. Methods used for selection of post-mitotic fibroblast cell types in-

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Table I. List of HDF Cell Populations Used as Feeder Layers

Fibroblast Cell Strain Tested[a]	Anatomic Site of Biopsy	Age of Donor (years)	Number of Passages
HF 290886	breast	25	10
HF 221087	forehead	74	5
HF 031187	temple	34	8
HF 71186	forehead	nk[b]	15
HF 030487	foreskin	2	14
HF 111187	foreskin	8	5
HF 310387	upper back	48	10
GM 0038A	presumably buttock	9	34
GM 1717	forearm	39	18

[a] "HF . . . ", HDF cell strains established in our laboratories and "GM . . . ", HDF cell lines obtained from the Human Genetic Mutant Cell Repository, Camden.
[b] nk: not known.

cluded mitomycin C and W-irradiation [18,19]. So far, attempts to use HDF as feeder cells were rarely reported and failed to document advantages compared to the 3T3-system [6,20,21]. The densities of these HDF-feeder layers ranged from 1.75×10^4 [20] to 2×10^4 cells/cm^2 [6,21], while their morphology was not described at all. Here we provide evidence that post-mitotic HDF are capable of sustaining growth of follicular and interfollicular keratinocytes efficiently and reproducibly, provided these feeder layers are prepared in an appropriate manner.

MATERIALS AND METHODS

Preparation of HDF-Feeder Layers Cultures of HDF were initiated from skin explants [22] of normal individuals of various age or obtained from the Human Genetic Mutant Cell Repository, Camden, NJ (see Table I).

Induction of Post-Mitotic State by Mitomycin C 8×10^3 HDF/cm^2 were incubated in DMEM containing 10% FCS and 8 μg/ml mitomycin C (Sigma) for 4 h at 37°C. Post-mitotic HDF obtained by this procedure were then rinsed 4 times with PBS, detached with 0.05% trypsin/0.02% EDTA, and replated at a density of $4-5 \times 10^3$ cells/cm^2 in DMEM supplemented with 10% FCS.

Induction of Post-Mitotic State by Irradiation Preconfluent cultures of HDF were trypsinized as mentioned above and the cells suspended at a cell density of 10^6/ml in DMEM with 10% FCS. Cryotubes containing 1–2 ml of this suspension were irradiated with a single dose of 7000 cGy (50 kV, 1.0 mm aluminum filter, distance 5 cm) using a Dermopan 2 (Siemens). Thereafter, the post-mitotic HDF were plated at a density of $4-5 \times 10^3$ cells/cm^2.

Interfollicular keratinocytes or ORS-cells were usually plated when the post-mitotic HDF had substantially increased their size, which normally happened 3–4 d after treatment (see *Results*). The HDF-feeder layers could be used for at least 4 weeks after preparation when incubated at 37°C.

Keratinocyte Isolation and Cultivation Isolation of ORS-cells from plucked HHF was carried out as described earlier [16]. Briefly, HHF were collected in DMEM buffered with 25 mM Hepes and supplemented with 400 U/ml penicillin and 400 μg/ml streptomycin. HHF in anagen phase were carefully selected under the dissecting microscope. In order to avoid any contamination with epidermal keratinocytes from the infundibulum or with trichocytes from the hair bulb, the infundibular part down to the presumptive level of the sebaceous gland as well as the bulbar portion were cut away. After two rinses in DMEM, the follicles were incubated with 0.1% trypsin (1:250) and 0.02% EDTA in PBS without Ca^{++} and Mg^{++} (pH 7.2) for 10 min at 37°C. A suspension essentially consisting of single cells was obtained by vigorously pipetting the follicles in DMEM supplemented with 10% newborn calf serum. After centrifugation for 10 min at 200 g, ORS-cells were resuspended in culture medium and seeded at a density of $0.5-1 \times 10^3$ cells/cm^2 on a HDF-feeder layer. Primary cultures were performed in FAD-medium composed of 3 parts DMEM and 1 part Ham's F12 supplemented with 10% FCS, insulin, hydrocortisone, adenine, triiodothyronine, choleratoxin, EGF, penicillin, and streptomycin [16,23,24]. Secondary cultures were grown either in MCDB 153-(Clonetics Corporation, San Diego, CA) or in FAD-medium.

Interfollicular keratinocytes were isolated from juvenile foreskin, and other surgical specimens from children and adults, by incubation with 0.25% trypsin in Ca^{++}- and Mg^{++}-free PBS (pH 7.2) for 1.5 h at 37°C. These cells were then cultured under the same conditions as the ORS-cells.

Keratin Analysis of Cultured ORS-Cells For metabolic labeling, confluent primary cultures were incubated with 10 μCi L-[^{35}S]methionine (NEN Chemicals, Zürich, Switzerland) per ml in methionine-free RPMI 1640 (Seromed, Basel, Switzerland) for 14 h at 37°C. The cells were then washed with PBS and thoroughly homogenized in 1% Triton X-100 containing low- and high-salt buffers (detailed methods are described in Refs 25 and 26). Cytoskeletal proteins were separated by one-dimensional (SDS) and two-dimensional (NEPHG/SDS) gel electrophoresis [27]. To detect labeled proteins, gels were stained with Coomassie blue, processed for fluorography using Enlightning (NEN), and exposed on Agfa Curix RP1 at −80°C.

RESULTS

HDF-Feeder Layers Systematic evaluation of several parameters for preparation of HDF-feeder layers revealed that the following conditions gave optimal results: induction of post-mitotic state by treatment either with 8 μg/ml mitomycin C for 4 h or irradiation with a single dose of 7000 cGy; subsequent plating density of $4-5 \times 10^3$ HDF/cm^2. After such treatments resumption of HDF proliferation was never observed. Moreover, such feeder layers could be stored for more than 4 weeks without loosing their feeder efficacy. Twenty-four hours after irradiation, HDF displayed a mostly spindle-like shape with long cytoplasmatic extensions (compare Fig 1*a* and *b*). However, these cells increased dramatically in size over the subsequent 5–10 d and finally acquired an essentially epitheloid, but pleiomorphic, shape (Fig 1*c*). In contrast, the mitomycin C-treated HDF changed to a flattened and epitheloid cell morphology (Fig 1*d*) already after 24 h with no significant changes later on, except further spreading (Fig 1*e*). This cellular enlargement had to be taken into account to determine the optimal HDF-density at the time of cell plating (Fig 1*c*,*e*). The post-mitotic HDF had to be allowed to fully spread out and acquire their final shape, but also to leave enough intercellular space for keratinocytes to nestle in between. $4-5 \times 10^3$ HDF/cm^2 turned out to fulfill these criteria while keeping an excellent feeder function. All the HDF-strains tested so far (Table I) exhibited the same morphologic changes after induction of the post-mitotic state, thus allowing consistent conditions in preparing the feeder layers. In particular, no striking differences were noted with respect to the anatomic site of skin biopsies and the number of passages already accomplished. Also, the efficiency in sustaining keratinocyte growth did not vary significantly. When needed, HDF-feeders could be selectively removed by 0.02% EDTA as effectively as 3T3-feeders.

Primary Keratinocyte Cultures on HDF-Feeder Layers Trypsinisation of plucked HHF yielded about $0.5-1 \times 10^4$ dispersed ORS-cells per follicle. Seeding of $0.5-1 \times 10^3$ ORS-cells/cm^2 on HDF-feeders prepared as described above resulted in a seeding efficiency of 4%–8% (n = 6). After 2–3 d in culture, single ORS-cells or groups thereof surrounded by post-mitotic HDF became visible. Subsequently, ORS-cell colonies enlarged continuously, pushing aside the surrounding feeder cells (Fig 2*a*). The proliferating ORS-cells were densely packed and displayed a typical epitheloid morphology with a high nuclear-cytoplasmic ratio (Fig 2*b*), which was essentially identical to that we previously reported on 3T3-feeders [16]. In accordance with Rheinwald and Green [6], we observed that in preconfluent cultures stratification occurred less frequently than on 3T3-feeders, a difference that was no longer apparent at confluency. In contrast to 3T3-feeders, however, HDF-

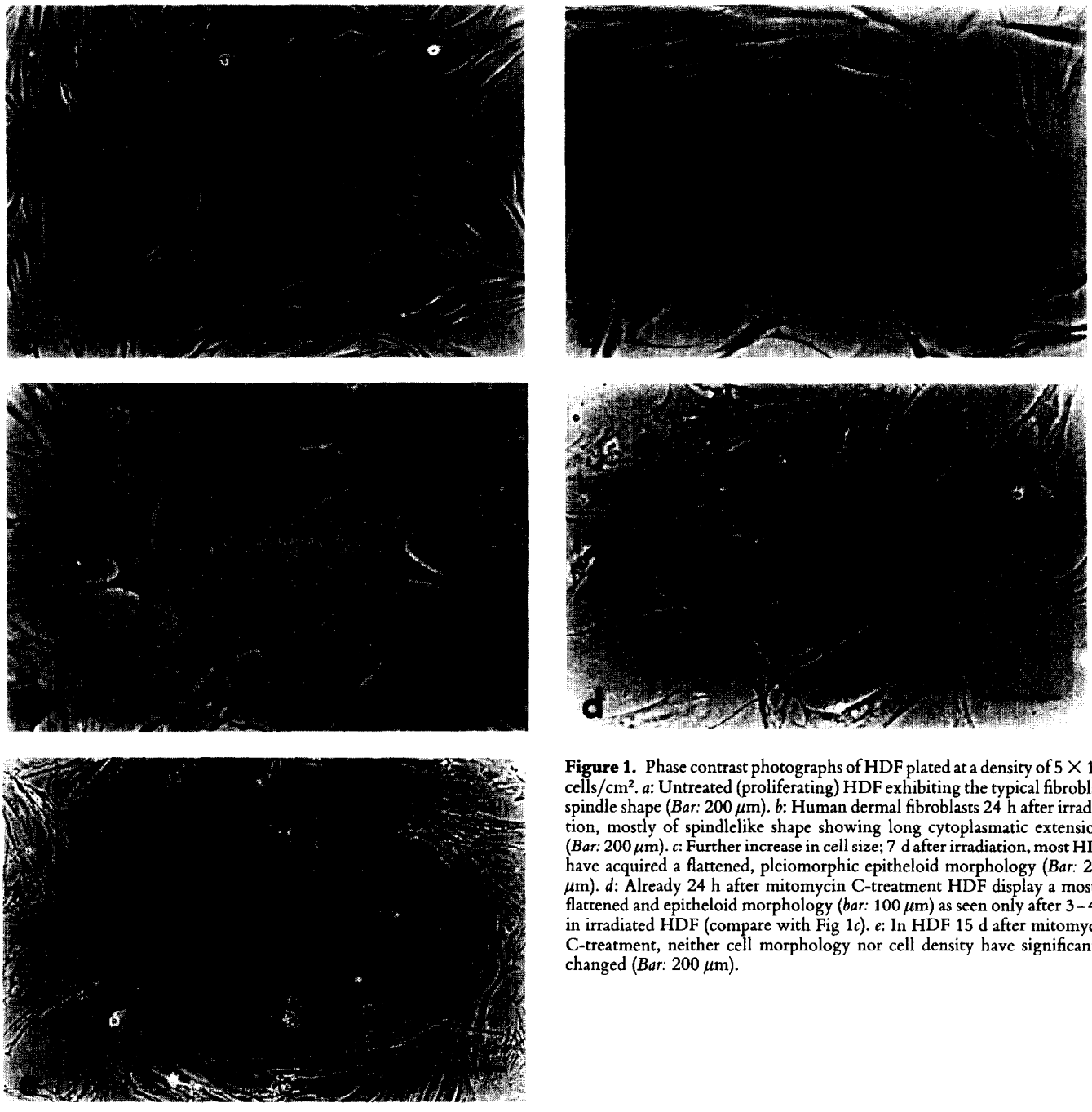

Figure 1. Phase contrast photographs of HDF plated at a density of 5×10^3 cells/cm^2. *a*: Untreated (proliferating) HDF exhibiting the typical fibroblast spindle shape (*Bar:* 200 μm). *b*: Human dermal fibroblasts 24 h after irradiation, mostly of spindlelike shape showing long cytoplasmatic extensions (*Bar:* 200 μm). *c*: Further increase in cell size; 7 d after irradiation, most HDF have acquired a flattened, pleiomorphic epitheloid morphology (*Bar:* 200 μm). *d*: Already 24 h after mitomycin C-treatment HDF display a mostly flattened and epitheloid morphology (*bar:* 100 μm) as seen only after 3–4 d in irradiated HDF (compare with Fig 1*c*). *e*: In HDF 15 d after mitomycin C-treatment, neither cell morphology nor cell density have significantly changed (*Bar:* 200 μm).

feeder layers never had to be renewed during the period necessary to obtain confluent cultures. On an average, this period lasted 3 weeks with little variation when different HDF-strains were tested. From confluent ORS-cultures an average of 1.0×10^5 cells/cm^2 ($n = 5$) was recovered. Assuming a seeding efficiency of 5%, this corresponds to 9.6 population doublings in the primary culture. Growth rates observed for ORS-cells using this new feeder technique are thus comparable to the best results in our previous studies with 3T3-feeder cells [16]. However, reproducibility was clearly enhanced in comparison to the 3T3-system as demonstrated by more than 60 primary ORS-cultures established so far on different HDF-feeder strains. Moreover, the handling of HDF-feeders avoids several problems often encountered with 3T3-cells such as (1) loss of feeder properties when regular passaging in preconfluent state is missed, (2) inconsistent response to growth-arrest inducing procedures, and (3) frequent degenerative changes and detachment requiring repeated renewal of feeder cells. Similar results with interfollicular keratinocytes on HDF-feeders indicate the wide applicability of this approach.

Keratinocyte Subcultivation Subcultivation was optimal when ORS-cells were still at a preconfluent stage. With a split-ratio of 1:3 (10^4 cells/cm^2), they grew readily even in the absence of a feeder layer, either in FAD- or in serum-free MCDB 153-medium. Under both conditions, ORS-cells could be successfully passaged 3 times. At clonal seeding densities ($< 10^2$ cells/cm^2), however, ORS-cells grew without feeders only in MCDB 153, but required HDF-feeders in FAD-medium.

Keratins Expressed in ORS-Cells Cultivated on HDF-Feeders Because keratins represent generally accepted markers of epithelial type and differentiation, their expression by ORS-cells grown on various HDF-feeders was analyzed. Irrespective of HDF-cells used as feeders, ORS-cells in primary and secondary cultures synthesized a 60-, 58-, 51-, and 49-kDa species (Fig 3*a*) in consistent

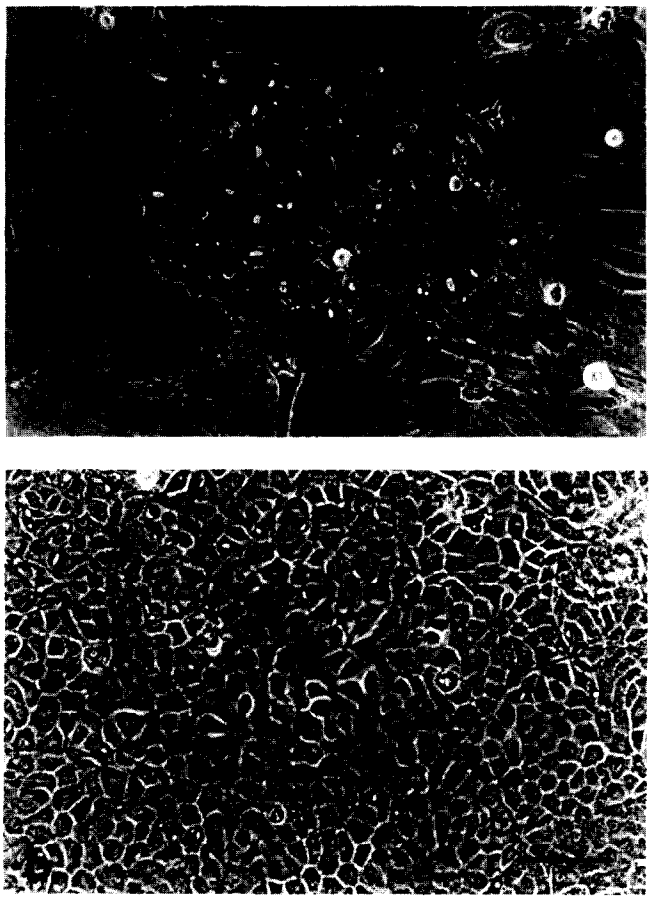

Figure 2. Phase contrast photographs of cultured ORS-cells in FAD-medium on HDF-feeders (primary culture; initial density 10^3 cells/cm^2). *a*: Growing ORS-cell colonies 6 d after plating pushing aside the enlarged mitomycin C-treated HDF (*Bar:* 100 μm). *b*: Confluent culture 3 weeks after plating (*Bar:* 100 μm).

ratios, corresponding to keratins nos. 5, 6, 14, and 16/17 (according to the human keratin catalog [28]). This pattern, also seen after labeling on fluorographs (Fig. 3*b*), was identical to that of cultures on 3T3-feeder cells and coincided largely with that expressed by ORS-cells in situ [27].

DISCUSSION

To establish primary cultures of interfollicular keratinocytes in the absence of a feeder layer, minimal plating densities were reported to range between 5×10^3 cells/cm^2 (neonatal foreskin, 5) and 1×10^4 cells/cm^2 (adult breast skin, 5). Attempts to initiate ORS-cell cultures with similar plating densities failed even when various substrata were tested (tissue culture plastic, extracellular matrix deposited by bovine corneal endothelial cells, fibronectin, type I-collagen, laminin, bovine eye lens capsules; our unpublished data). In contrast, on 3T3-feeders, dispersed ORS-cells in primary cultures grew readily even when seeded at densities of only $2-3 \times 10^3$ cells/cm^2 [16]. In subsequent experiments, the reproducibility of these results was hampered by major problems with the 3T3-feeder technique. Alternatively, using post-mitotic human dermal fibroblasts obtained either by irradiation or mitomycin C-treatment, we were able to avoid the technical problems. Under the conditions used, the HDF-feeders regularly supported growth of follicular and also interfollicular keratinocytes as efficiently as only occasionally observed with 3T3-feeders. On average, the seeding efficiency of primary ORS-cell cultures was 4%–8% with HDF-feeders compared to 2%–3% previously found with 3T3-feeders [16]. Optimal plating densities for the HDF-feeder strain tested so far ranged between $4-5 \times 10^3$ cells/cm^2. This does not exclude that other HDF-strains may require slightly different experimental conditions.

A crucial point using this new feeder technique is the proposed plating density: 1) for settling of the epithelial cells some freely available substratum seems to be absolutely required and 2) the expansion of the epithelial colonies should not be impeded by too densely packed HDF-feeder cells. This was particularly important when fresh (up to 7 d old) HDF-feeders were used, because at this stage these cells still showed significant spreading. The failure of earlier reports to document advantages of HDF-feeders compared to the 3T3-technique [6,20] might thus be explained by feeder cell densities exceeding 2×10^4/cm^2.

Keratin expression of ORS-cells co-cultured with HDF-feeders was not modified in comparison to ORS-cells cultured on plastic or with 3T3-feeders, and very similar to the pattern seen in situ [27]. Also, in terms of quantity, the "basal" keratins no. 5 and no. 14, and the "hyperproliferative" keratins no. 6 and no. 16, commonly expressed in hyperplastic epidermis [29] and in cultures of interfollicular keratinocytes [1,27,30], were synthesized at comparable ratios. Thus, based on keratin analysis as a biochemical differentiation marker, primary ORS-cell cultures exhibited a similar state of differentiation independent of the feeder system applied.

In conclusion, the presented HDF-feeder technique seems to provide the following advantages: 1) easier handling and better

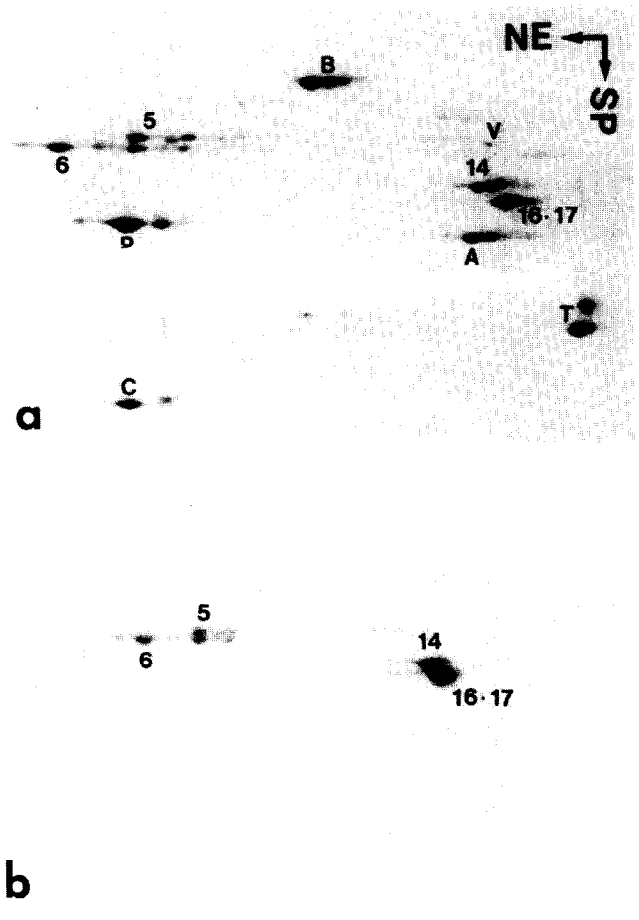

Figure 3. Two-dimensional gel electrophoresis of cytoskeletal proteins (NEPHG/SDS gels) of keratins expressed in ORS-cells grown on HDF-feeder cells. After 22 d in primary culture, proteins of cells were labeled (14 h) with [^{35}S]-methionine. *a*: Coomassie staining; *b*: corresponding fluorograph (*v*; presumably vimentin from residual HDF-cells). Standard proteins were *B*: bovine serum albumin (68 kDa); *P*: phosphoglycerate kinase (43 kDa); *A*: α-actinin (42 kDa); *T*: tropomyosin (36 and 38 kDa); *C*: carbonic anhydrase (30 kDa). Migration in the first dimension NEPHG *(NE)* was from right to left (basic end). In the second dimension SPAGE *(SP)* was applied.

reproducibility because they are less delicate than those made up of 3T3-cells; 2) the possibility to store HDF-feeders for at least 4 weeks without detectable loss of feeder efficacy; 3) the choice to use HDF from specific anatomic sites and over a large range of cumulative population doublings; and 4) the use of initially diploid human dermal fibroblast strains instead of aneuploid, ill-defined transformed mouse cell lines. In view of potential immunologic and infectious (viral) hazards, feeder cells from the same species are preferable to establish keratinocyte cultures for wound covering in humans. Of course, repeated serologic controls of donors have to be conducted to rule out potential contamination with HIV- and hepatitis B-viruses.

Finally, although the exact mechanisms underlying the conditioning effect of feeder cells still remain obscure, this co-culture system could provide a valuable tool for the study of mesenchyme-epithelial interactions in vitro. Apart from avoiding species differences there would be the option of using cells from specific anatomic sites and of known donor age. In particular, such investigations could be relevant if irradiation or mitomycin C-treatment do not merely lead to mitotic arrest of HDF, but switch these cells towards discrete post-mitotic levels of terminal differentiation, as proposed by Bayreuther et al [17–19]. So far, a similar approach did not reveal any differential effects between mitomycin C-treated fibroblasts from psoriatic and normal individuals on epidermal keratinocytes in classical submerged cultures [31]. Future work, however, planned in more elaborate culture systems, might shed light on interactions between various cutaneous epithelial cells and dermal fibroblasts from normal and diseased skin.

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REFERENCES

1. Fusenig NE: Mammalian epidermal cells in culture. In: Bereiter-Hahn J, Matoltsy AG, Silvia Richards K (eds.). Biology of the Integument, vol 2. Berlin, Springer-Verlag, 1986, pp 409–442
2. Liu SC, Karasek M: Isolation and growth of adult human epidermal keratinocytes in cell culture. J Invest Dermatol 71:157–162, 1978
3. Gilchrest BA, Nemore RE, Maciag T: Growth of human keratinocytes on fibronectin-coated plates. Cell Biol Int Rep 4:1109–1016, 1980
4. Boyce ST, Ham RG: Calcium-regulated differentiation of normal human epidermal keratinocytes in chemically defined clonal culture and serum-free serial culture. J Invest Dermatol 81(suppl):33s–40s, 1983
5. Wille JJ, Pittelkow MR, Shipley GD, Scott RE: Integrated control of growth and differentiation of normal human prokeratinocytes cultured in serum-free medium: clonal analyses, growth kinetics, and cell cycle studies. J Cell Physiol 121:31–44, 1984
6. Rheinwald JG, Green H: Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell 6:331–344, 1975
7. Gallico GG, O'Connor NE, Compton CC, Kehinde O, Green H: Permanent coverage of large burn wounds with autologous cultured human epithelium. N Engl J Med 311:448–451, 1984
8. Green H, Kehinde O, Thomas J: Growth of cultured human epidermal cells into multiple epithelia suitable for grafting. Proc Natl Acad Sci USA 76:5665–5668, 1979
9. Oliver RF: Ectopic regeneration of whiskers in the hooded rat from implanted lengths of vibrissa follicle wall. J Embryol exp Morph 17:27–34, 1967
10. Oliver RF: The experimental induction of whisker growth in the hooded rat by implantation of dermal papillae. J Embryol exp Morph 18:43–51, 1967
11. Jahoda CAB, Horne KA, Oliver RF: Induction of hair growth by implantation of cultured dermal papilla cells. Nature 311:560–562, 1984
12. Eisen AZ, Holyoke JB, Lobitz WC: Responses of the superficial portion of the human pilosebaceous apparatus to controlled injury. J Invest Dermatol 25:145–156, 1955
13. Weterings PJJM, Vermorken AJM, Bloemendal H: A method for culturing human hair follicle cells. Br J Dermatol 104:1–5, 1981
14. Imcke E, Mayer-da-Silva A, Detmar M, Tiel H, Stadler R, Orfanos CE: Growth of human hair follicle keratinocytes in vitro. J Am Acad Dermatol 17:779–786, 1987
15. Wells J: A simple technique for establishing cultures of epithelial cells. Br J Dermatol 107:481–482, 1982
16. Limat A, Noser FK: Serial cultivation of single keratinocytes from the outer root sheath of human scalp hair follicles. J Invest Dermatol 87:485–488, 1986
17. Bayreuther K, Rodemann HP, Hommel R, Dittmann K, Albiez M, Francz P: Human skin fibroblasts in vitro differentiate along a terminal cell lineage. Proc Natl Acad Sci USA 85:5112–5116, 1988
18. Francz PI, Bayreuther K, Rodemann HP, Cytoplasm-, nuclear-, membrane bound-, and secreted (35S)- methionine polypeptide pattern in differentiating fibroblast stem cells in vitro. J Cell Science (in press)
19. Rodemann HP, Bayreuther K, Francz PI, Dittmann K, Albiez M: Selective enrichment and biochemical characterization of seven human skin fibroblasts cell types in vitro. Exp Cell Res 180:84–93, 1989
20. Kubilus J, Macdonald MJ, Baden HP: Epidermal proteins of cultured human and bovine keratinocytes. Biochim Biophys Acta 578:484–492, 1979
21. Rheinwald JG: Serial cultivation of normal human epidermal keratinocytes. In: Harris CC, Trump BF, Stoner GD (eds.). Methods in Cell Biology, vol. 21A. New York, Academic Press, 1980, pp 229–254
22. Sly WS, Grubb J: Isolation of fibroblasts from patients. In: Jakoby WB, Pastan IH (eds.). Methods in Enzymology, vol. 58. New York, Academic Press, 1979, pp 444–450
23. Gilfix BM, Green H: Bioassay of retinoids using cultured human conjunctival keratinocytes. J Cell Physiol 116:172–174, 1984
24. Wu YJ, Parker LM, Binder NE, Beckett MA, Sinard JH, Griffiths CT, Rheinwald JG: The mesothelial keratins: a new family of cytoskeletal proteins identified in cultured mesothelial cells and nonkeratinizing epithelia. Cell 31:693–703, 1982
25. Breitkreutz D, Bohnert A, Herzmann E, Bowden PE, Boukamp P, Fusenig NE: Differentiation specific functions in cultured and transplanted mouse keratinocytes: environmental influences on ultrastructure and keratin expression. Differentiation 26:154–169, 1984
26. Breitkreutz D, Hornung J, Pöhlmann J, Brown-Biermann L, Bohnert A, Bowden PE, Fusenig NE: Environmental induction of differentiation specific keratins in malignant mouse keratinocyte lines. Eur J Cell Biol 42:255–267, 1986
27. Stark HJ, Breitkreutz D, Limat A, Bowden P, Fusenig NE: Keratins of the human hair follicle: "hyperproliferative" keratins consistently expressed in outer root sheath cells in vivo and in vitro. Differentiation 35:236–248, 1987
28. Moll R, Franke WW, Schiller DL: The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 31:11–24, 1982
29. Weiss R, Eichner R, Sun TT: Monoclonal antibody analysis of keratin expression in epidermal diseases: a 48- and 56-Kdalton keratin as molecular markers for hyperproliferative keratinocytes. J Cell Biol 98:1397–1406, 1984
30. Sun TT, Eichner R, Schermer A, Cooper D, Nelson WG, Weiss RA: Classification, expression and possible mechanisms of evolution of mammalian epithelial keratins: a unifying model. In: Lewine A, Van de Wonde GF, Torpe WE, Watson JD (eds.). Cancer Cells, Vol. 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, 1984, pp 169–176
31. Baden HP, Kubilus J, Macdonald MJ: Normal and psoriatic keratinocytes and fibroblasts compared in culture. J Invest Dermatol 76:53–55, 1981