Expression of dermal extracellular matrix proteins in a newly developed full-thickness skin model

Mewes, K. R.[1], Zoeller, N. N.[1,2], Bernd, A.[2], Prießner, A.[1], De Wever, B.[1], Schröder, K. R.[1]

[1] Phenion GmbH & Co. KG, Merowingerplatz 1a, 40225 Düsseldorf, Germany,
[2] Dept. of Dermatology and Venerology, University Hospital, Frankfurt/Main, Germany
contact: karsten.mewes@henkel.com, phone: +49-211-797-4593

Introduction

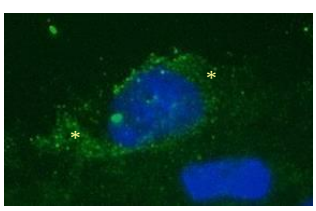
The dermal elastic fibres of the human skin are mainly composed of elastin and fibrillin-1. They can be distinguished according to their elastin-fibrillin ratio, their length and their orientation in respect to the dermo-epidermal junction zone (DEJ).
Fibulin-5 (*aka* DANCE/EVEC), an integrin- and calcium-binding extracellular matrix protein, can link these fibres to the cells of the connective tissue.
In order to study the development and the properties of the elastic network *in vitro*, we analyzed the expression of elastin, fibrillin-1 and fibulin-5 in a newly developed full-thickness skin equivalent. In addition we were interested in the synthesis and secretion of matrix metalloproteinases (MMPs). These proteolytic enzymes can degrade ECM proteins and thus contribute to the turnover of the connective tissue.

Methods

Production of the skin model:
The skin equivalents consisted of a lyophilized collagen matrix and fibroblasts and keratinocytes isolated from human foreskin tissue. After a 3 weeks cultivation period under submersed conditions the skin models were cultivated at the air-liquid interface (ALI) for another 2 weeks.

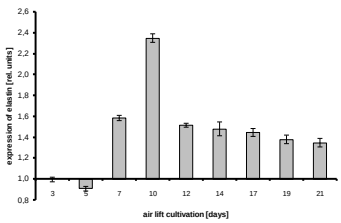
Immunohistochemistry:
Cryosections (8 µm) were fixed in ice-cold acetone, washed with TBS, blocked with 10% normal goat serum (NGS) in phosphate-buffered saline, and incubated with the 1st antibody. The sections were washed again and incubated with the 2nd antibody, coupled to Alexa Fluor 488® and 568®, resp. Both antibody solutions contained 10% NGS for effective background reduction. Nuclei were counterstained with DAPI (2 µg/ml).

MMP-2 expression

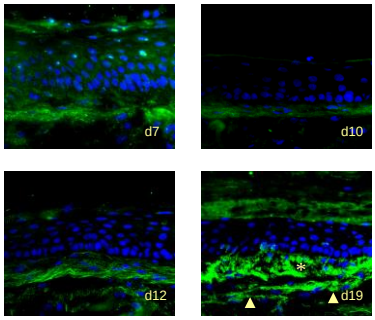


MMP-2 (gelatinase A) can be detected in the cytoplasm of fibroblasts in the dermal compartment of the full-thickness skin model.
Immunostaining on cryosections with MMP-2 antibody (green, asterisks); nuclei counterstained with DAPI (blue).

Elastin expression

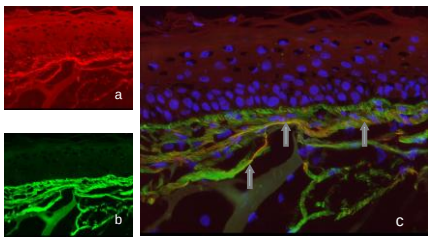


Elastin expression, based on the amount of elastin mRNA extracted from full-thickness skin models, starts at day 7 of the air-liquid interface (ALI), increases markedly to a maximum level at day 10. After a decrease at day 12 the mRNA level remains rather constant throughtout the culture period.
Gene expression was determined with realtime-RT-PCR using the indicated primers: forward: 5'-GCT AAG GCA GCC AAG TAT GG-3', reverse: 5'-CAG CTC CAA CCC CGT AAG TA-3'



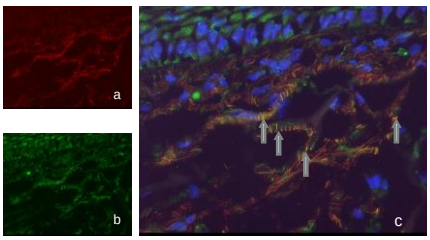
With increasing culture time (days 7-19) elastin-positive fibres accumulate in the dermis of the skin model. First appearance of elastin-positive material at day 7 of ALI.
Short, perpendicularly to the DEJ oriented fibres dominate in the upper dermis [*], longer, more parallely oriented fibres are found in the deeper dermal regions [▲].
Immunostaining with anti-elastin antibody (green); nuclei counterstained with DAPI (blue).

Fibrillin-1 expression



Fibrillin-1 and **elastin** can be co-localized on the fibres of the elastic system, which clearly matches the biochemical composition of these fibres found in human skin.
Again, regional differences in length and orientation of the elastic fibres can be seen.
Immunostaining with anti-elastin (red, **a**) and anti-fibrillin antibody (green, **b**); nuclei counterstained with DAPI (blue). **c**) overlay picture
arrows: double-stained fibres

Fibulin-5 expression



Fibulin-5 and **elastin** can be co-localized on the fibres of the elastic system, which clearly matches the chemical composition of these fibres found in human skin. Again, regional differences in length and orientation of the elastic fibres can be seen.
Immunostaining with anti-elastin (red, **a**) and anti-fibulin antibody (green, **b**); nuclei counterstained with DAPI (blue). **c**) overlay picture
arrows: double-stained fibres

Conclusions

- Main components of the dermal elastic network, elastin, fibrillin-1 and fibulin-5, are expressed in the skin equivalent.
- These proteins can be co-localized on individual fibres, thus confirming the biochemical composition and the correct assembly of the elastic network found *in vivo*.
- Length and orientation of the elastic fibres differ due to their location in the dermis.
- As one example of the MMP family, MMP-2 (gelatinase A), can be detected in the dermal fibroblasts.
- **These results suggest our skin equivalent to be a well-suited tool to study the dermal elastogenesis *in vitro*.**