Studying dermal elastogenesis in a full-thickness skin equivalent

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Introduction

It is assumed that Niccolò Paganini, the famous Italian musician, was able to play the violin with unprecedented virtuosity only because suffering from a connective tissue disorder which resulted in increased joint flexibility, particularly in the upper limbs. Two disorders are under discussion. In the Ehlers-Danlos syndrome the collagen network is severely affected. The Marfan syndrome, in contrast, is often linked to a mutant fibrillin-1 gene, thus affecting the microfibrils and consequentially the elastic fibers in several human tissues including the blood vessel and the skin.

The dermal elastic fibers of the human skin are mainly composed of elastin and fibrillin-1 and 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 fibers to the cells of the connective tissue and is known to play a major role in the assembly of the elastic network. Dermal extracellular collageneous and elastic fibres are subject to degradation by matrix metalloproteinases (MMPs) due to intrinsic and extrinsic stress situations.

Today there is an increasing need for innovative animal-free test systems in order to study skin physiology and pathology *in vitro*. 3D-reconstructed skin models can offer such a promising alternative.

Aim

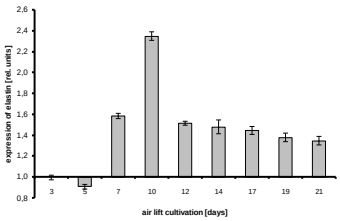
It is our aim to establish a cell-based research tool in order to study the development of the elastic network as well as the genesis of its disorders *in vitro*. Therefore we analyzed the expression of **elastin**, **fibrillin-1, fibulin-5** and of **MMPs** in a newly developed full-thickness skin model.

Methods

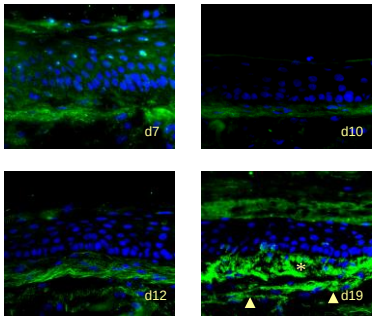
Production of the skin model:
The skin equivalents consisted of a lyophilized collagen matrix and fibroblasts and keratinocytes isolated from human foreskin tissue. After a 3 weeks cultivation period under submersed conditions the skin models were cultivated at the air-liquid interface (ALI) for another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

Elastin expression

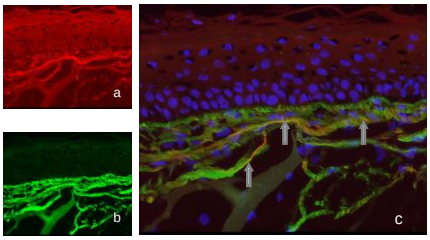


Elastin expression, based on the amount of elastin mRNA extracted from full-thickness skin models, starts at day 7 of the air-liquid interface (ALI), increases markedly to a maximum level at day 10. After a decrease at day 12 the mRNA level remains rather constant throughout the culture period.
Gene 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'



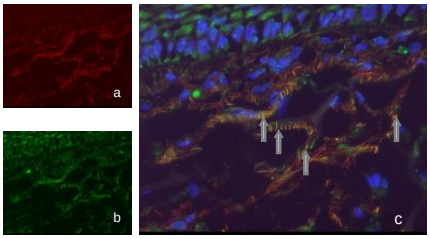
With increasing culture time (days 7-19) elastin-positive fibers accumulate in the dermis of the skin model. First appearance of elastin-positive material at day 7 of ALI. Short, perpendicularly to the DEJ oriented fibres dominate in the upper dermis [*], longer, more parallely oriented fibers are found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

Fibrillin-1 expression



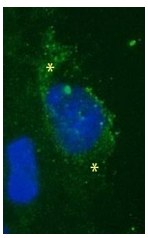
Fibrillin-1 and **elastin** can be co-localized on the fibers of the elastic system, which clearly matches the biochemical composition of these fibers found in human skin.
Again, regional differences in length and orientation of the elastic fibers can be seen.
Immunostaining with anti-elastin (red, **a**)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

Fibulin-5 expression



Fibulin-5 and **elastin** can be co-localized on the fibers 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 fibers 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 fibers

MMP-2 expression



MMP-2 (gelatinase A) can be detected, scattered throughout the cytoplasm of the 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).

Conclusions

Main components of the dermal elastic network, elastin, fibrillin-1 and fibulin-5, are expressed in the skin equivalent and can be co-localized on individual fibers.

Length and orientation of the elastic fibers differ due to their location in the dermis.

As one example of the MMP family, MMP-2 can be detected in the dermal fibroblasts.

These results suggest our skin equivalent to be a promising tool to study elastogenesis and dermal diseases *in vitro*.