STUDY OF THE DERMAL-EPIDERMAL JUNCTION ORGANIZATION AND MECHANICAL PROPERTIES BY ATOMIC FORCE MICROSCOPY

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THE DEJ IS A HIGHLY ORGANIZED STRUCTURE

The dermal-epidermal junction (DEJ) is a complex and highly organized structure (figure 1), which primary function is to anchor the epidermis to the dermis. The DEJ thus preserves skin integrity and participates to its mechanical properties. It also has an important metabolic role as it controls exchanges between epidermis and dermis (water, nutrients, growth factors,…). Atomic Force Microscopy (AFM) is an innovative technique that enables to determine DEJ molecular organization and mechanical properties (mechano organization al analysis).

All studies were conducted on human skin biopsies obtained from young or aged donors.

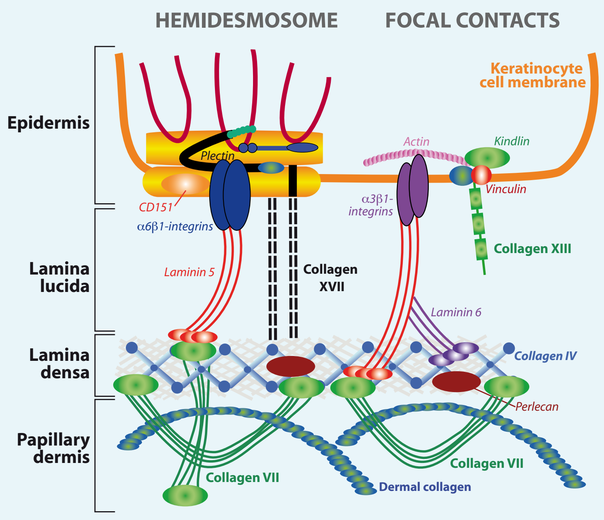
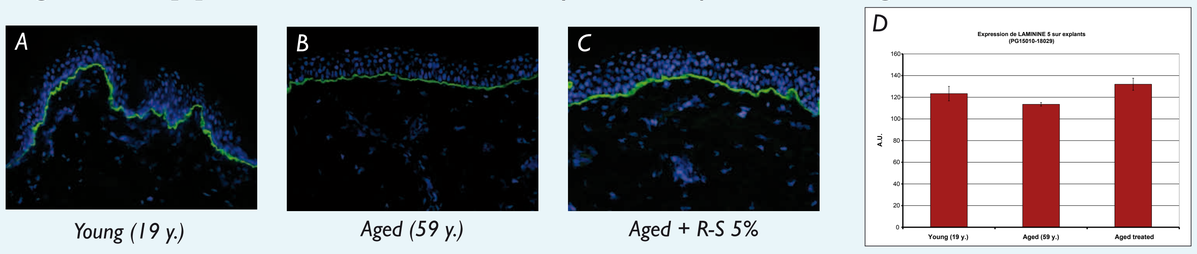


Figure 1: schematic of molecular arrangements within the DEJ

AGING INDUCES IMPORTANT MODIFICATIONS IN THE DEJ

Intrinsic / extrinsic aging is associated with ultra-structural modifications including flattening (loss of rete ridges), duplication, thinning and even partial disruption [**1**]. These changes likely are responsible for elasticity loss, higher fragility, decreased resilience to stress. Metabolic consequences are multiple, including dryness [**2**] and age spots formation [**3**], [**6**]. At the molecular level, there is a decreased expression of DEJ proteins [**4**] such as collagens type IV, VII and XVII, laminin-5, or integrin ß4. To improve DEJ in aged skin, we have designed an original active ingredient (R-S) that stimulates DEJ proteins expression and improves DEJ organization [**5**]. Enhancement of laminin 5 synthesis is presented in figure 2.



Young (19 y.) *Aged (59 y.)* *Aged + R-S 5%*

Figure 2: monitoring of laminin-5 (in green) within the DEJ of human skin explants obtained from A) a 19 year-old donor, B) a 59 year-old donor, C) the 59-year old explant treated with 5% R-S, in blue, staining of nuclei D) Quantification from image analysis.

THE AFM TECHNOLOGY

Topographical analysis with AFM

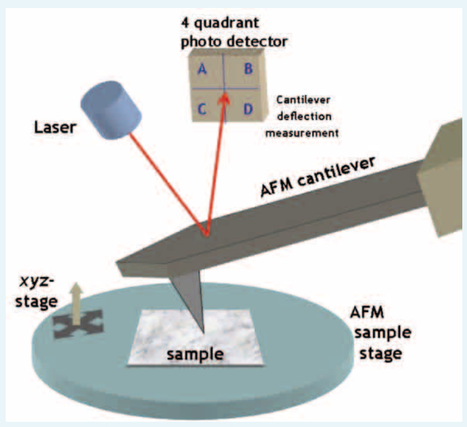
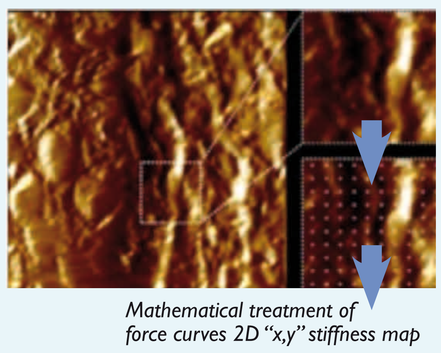


Figure 3: schematic of a typical Atomic Force Microscopy experimental setup

AFM is a scanning probe microscopy technique that was primarily designed for nanoscale 3D surface profiling. It consists in a very sharp tip (the probe) supported by a flexible cantilever, that scans the surface of the sample (figure 3). Cantilever oscillations are recorded with a laser beam/ photodiodes setup. The topography of the sample surface is reconstructed from the data collected by the photodetector.

Mechano-organizational analysis with AFM force spectroscopy



Mathematical treatment of force curves 2D "x,y" stiffness map

Figure 5: representation of the surface analysed by AFM "force microscopy" (the DEJ and adjacent epidermis and dermis). Each dot represents one indentation

In this particular AFM mode, the probe regularly indents the substrate (repeatedly applies a pressure on the sample). This "tapping mode" provides information on the interior of the sample (the probe deepens into the sample). 100 indentations were done over a surface covering the DEJ and adjacent zones (figure 5). Mathematical processing of "force-distance" curves gives a modulus of elasticity (Ea) that can be used for stiffness tomography of biological samples.

Results from AFM topographical analysis

Scanning of a skin section reveals that the DEJ is bulging at the surface of the cut (figure 4) due to a better water retention. Although precise topographical data on sections have no biological significance, it comes that DEJ is a well-delimited region. This enables an accurate measurement of DEJ thickness. In agreement with other reports, this technique shows that aging is associated with a thinning of the DEJ. Treatment with 5% R-S results in a thickened DEJ.

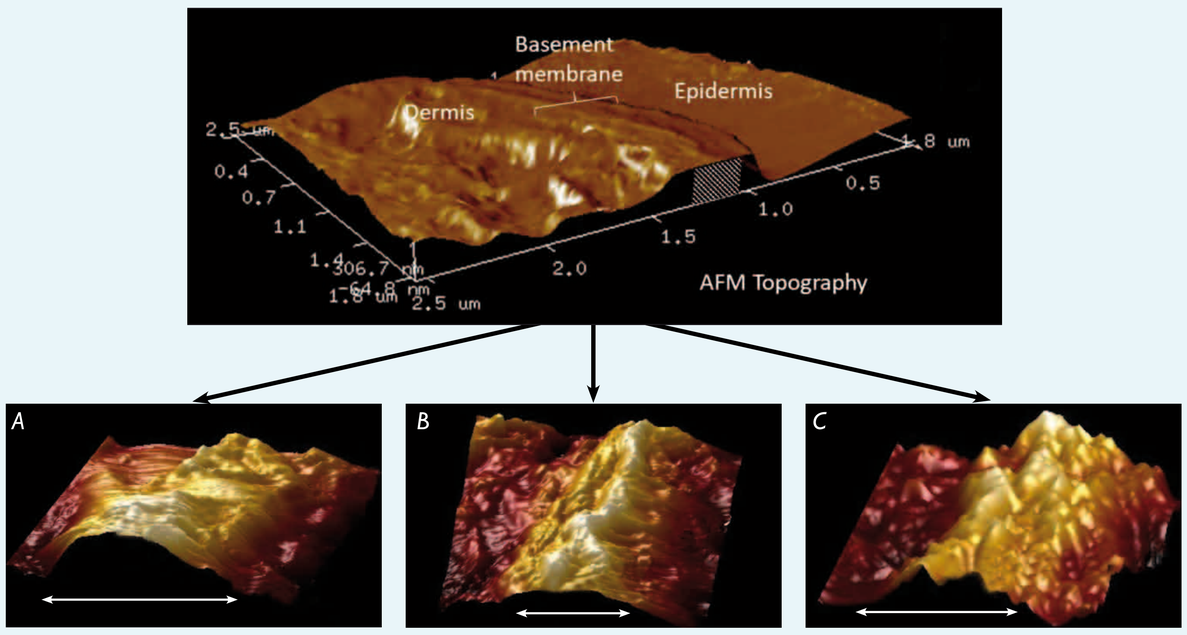


Figure 4: 3D topographical images obtained with AFM
A) section coming from a young donor (31 y.),
B) section coming from an aged donor (59 y.),
C) section coming from an aged donor treated with 5% R-S,
D) measurement of DEJ's thickness,
Above: a 3D representation of the scanned surface

D	Young	Aged	Aged treated
Thickness (µm)	1.104 ± 0.176	0.665 ± 0.031	0.856 ± 0.181

Results 2D "X,Y" stiffness maps of young and aged biopsies

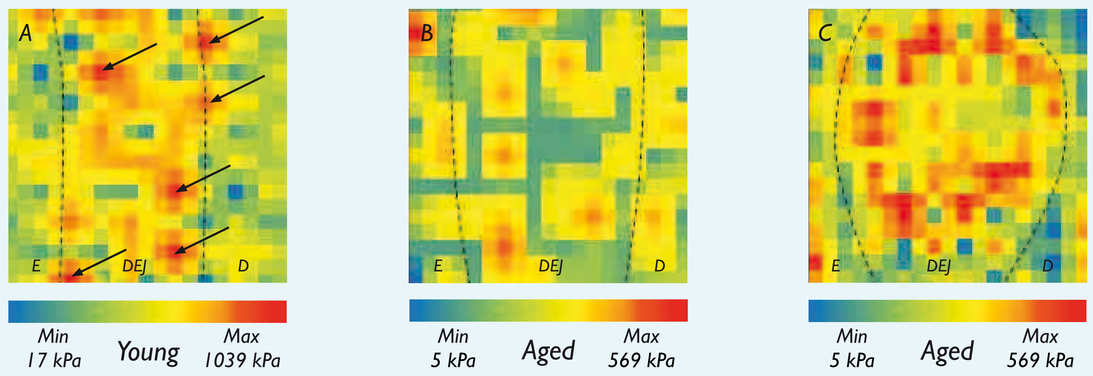


Figure 6: representative "x,y" stiffness maps obtained from A) young skin biopsies (31 year-old donor; no photoaging) B) an explant obtained from a photo-aged skin (59 year-old donor), C) another site from the photo-aged explants. Dot lines separate epidermis (E) /DEJ/dermis (D)

1- YOUNG

- DEJ in young skin (A) is a rigid zone (more than adjacent epidermis/dermis).
- DEJ from young skin is a well-organized structure, with a regular alignment of "strongpoints" (arrows in A).

2- AGED

- "Aged" DEJ (B) is loose (compare scales), and completely disorganized.
- "Aged" DEJ shows localized rigid structures (C), probably aggregates of non-organized DEJ proteins
- Functional loss in aged skin is also noticeable in adjacent zones, that are loose too.

Results 2D "X,Y" stiffness map of an aged biopsy treated with R-S

DEJ rigidity / organization is not completely restored by treatment (figure 7), however DEJ becomes homogenous and alignments of strong points in the epidermis suggest that re-attachment between DEJ and epidermis is initiated. Both observations suggest that DEJ re-organization has begun in treated aged biopsies. Interestingly, no aggregates were detected in treated aged biopsies. To verify the absence of aggregates, we recorded a "z-y" stiffness map (indentations are done all along the DEJ => see figure 8).

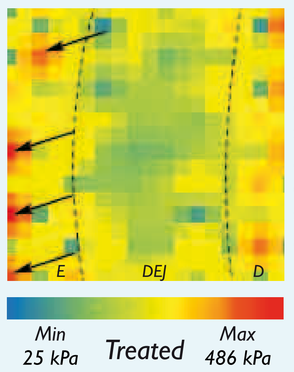


Figure 7: representative "x,y" stiffness map obtained from a photo-aged skin (59 y.) treated 48 hours with R-S 5%. Arrows point on a characteristic alignment of strongpoints in the epidermis (attachment sites)

Results Improved aggregates detection with "Z,Y" stiffness maps

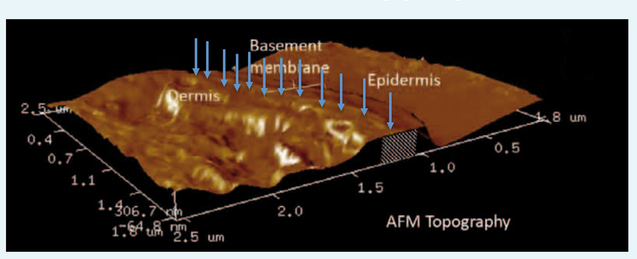
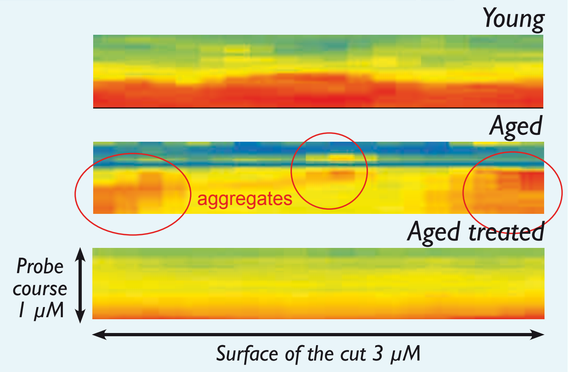


Figure 8: representative "z-y" stiffness maps (see blue arrows: indentations are done along the DEJ) from a young skin (31 year-old), a photo-aged skin (59 year-old donor), and the same aged skin treated 48 hours with R-S 5%.



This analysis was done all along the DEJ (figure 8), and mainly brings information on its mechanical homogeneity (for details see abstract):

- The DEJ of the young donor is rather homogeneous (smooth at the section's surface; more rigid as the probe deepens in the biopsy).
- The DEJ of the aged donor is heterogeneous. During its course, the probe met some rigid structures, e.g. aggregates. The sample is also much looser.
- The DEJ of the aged donor treated with 5% R-S is evenly organized (although it remains looser than the young skin). Absence of aggregates formation is confirmed.

CONCLUSION

AFM analysis brings valuable information on DEJ organization and mechanical properties. It shows that aging profoundly disorganizes DEJ which results in a functional failure (DEJ and adjacent skin become much looser). In line with our previous findings with other techniques, we see that R-S improves DEJ organization. Upon treatment with R-S, DEJ becomes homogeneous with the disappearance of aggregates characteristic of aged skin. Adjacent skin layers become more rigid.

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