Context

Skin is the largest organ in the human body and represents our first barrier against external stress but also undergoes internal stress, such as intrinsic aging. In this study, we decided to investigate the epidermis, a pluristratified tissue composed of several layers of keratinocytes harboring different states of differentiation. The deeper layer of keratinocytes, called basal layer, is responsible for maintaining tissue homeostasis including regeneration and renewal of the epidermis. Indeed, this one is in perpetual renewal thanks to the natural process of desquamation or regeneration, in case of injury. The keratinocytes from basal layer containing putative interfollicular stem cells asymmetrically divide. This allows to both maintain a pool of stem cells and provide cells intended for the upper layers of epidermis which will go through several steps of differentiation. The ultimate step of differentiation provides corneocytes which will be eliminated during the process of desquamation. Hence, these cells, from the basal layer, have an essential role in tissue homeostasis.

These basal cells are located on the dermal-epidermal junction involved in maintaining the association of the epidermis with the dermis and allowing exchanges between these two compartments. The dermis mainly made up of fibroblasts, collagen, and elastin fibers, plays a role in both physical and nutritional support of the epidermis. Collagen and elastin fibers respectively bring to the skin, resistance, and elasticity, while the blood microvascularization network allows dermis and epidermis nutrition. The dermal-epidermal junction, characterized by the expression of proteins such as collagen IV, or laminin 332, is composed of epidermal rete-ridges and dermal papilla, forming undulations. These undulations attenuate during aging and the dermal-epidermal junction becomes flatter. Basal cell layer located on the dermal-epidermal junction has been described to contain several cell subpopulations such as transit amplifying cells, undifferentiated keratinocytes and putative interfollicular stem cells. These last ones are important for epidermal renewal. They locate on dermal papillae and express specific markers such as MSCP protein **(Figure 1).**

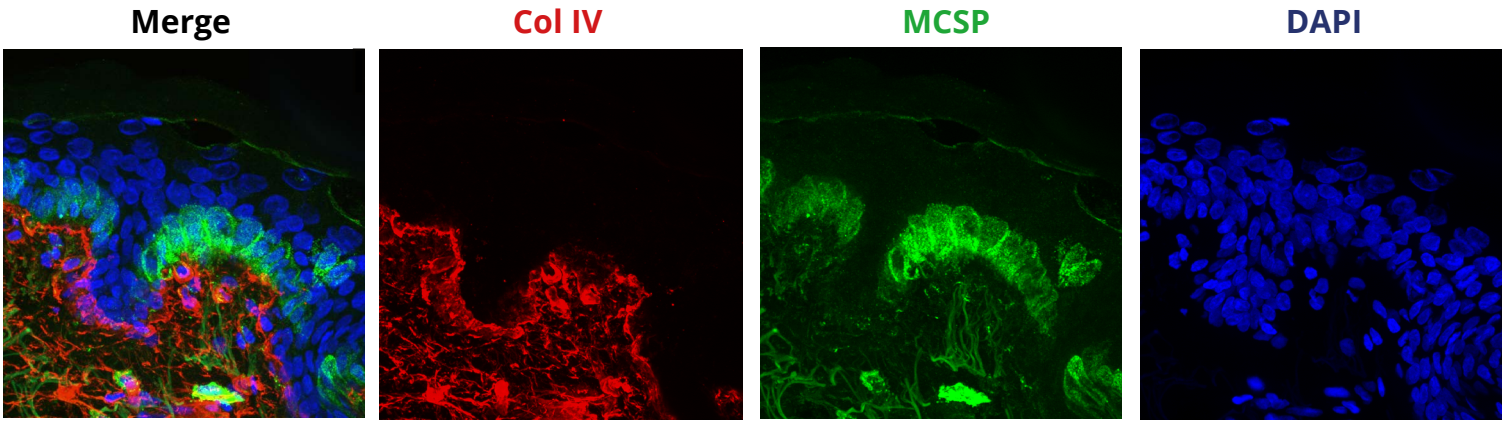

Figure 1: *Localization of MCSP protein in cell subpopulations of epidermal basal layer from skin tissue sections of 42 years old donor, stained for Col IV (red), MCSP (green) and DAPI (blue).*

Why use atomic force microscopy **to study anti-aging and regenerating effect of an active ingredient?**

During aging, dermal-epidermal junction flattens, the expression of MCSP protein (or other specific markers) decreases, that is concomitant with a decrease in the renewal rate and a reduction in the epidermis thickness. Then, the very specific interface between epidermis and dermis, including the dermal-epidermal junction, undergoes a remodeling leading to modifications in cell proliferation and in cell fate.

Performing stiffness mapping of basal cells and dermal-epidermal junction, makes possible to evaluate the evolution and modifications of mechanical properties of this specific area during aging **(Figure 2)**. Moreover, this approach provides new tools for analyzing and characterizing the efficiency of an active ingredient, claiming anti-aging or regenerative effects.

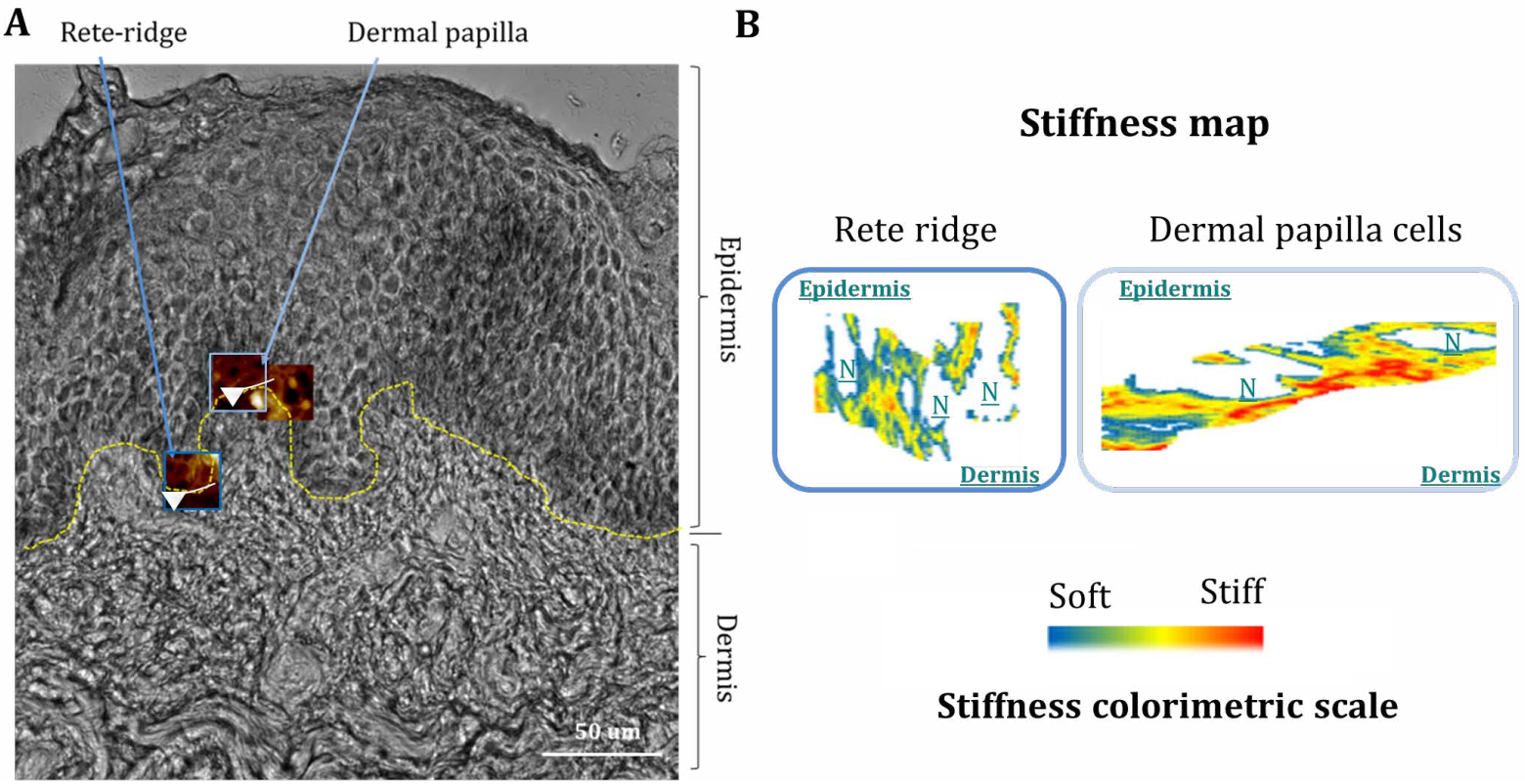

Figure 2: *Stiffness map of cell subpopulations anchored on the dermal-epidermal junction. A) Brightfield image showing lateral section of abdominal skin revealing DEJ (yellow dotted lines) or rete ridge cells (dark blue box) or dermal papillae cells (light blue box). On these cells are performed AFM measurements (AFM tips in white). Scale bar = 50µm. B) Stiffness map performed by atomic force microscopy on basal cells and more precisely on cells either from rete ridge or dermal papilla . N: Nucleus.*

Results

It has been described that a specific mechanical environment or a biomechanical state of cells could bring information on cell fate. Based on this information, we investigated the mechanical properties of basal cell subpopulations, and we measured the stiffness mapping of cells located above the dermal epidermal junction.

We therefore assessed by atomic force microscopy (AFM) stiffness properties of different cell subpopulations of the basal layer. More specifically, we analyzed if stiffness of cells expressing MCSP protein described as putative stem cells and located on dermal papilla were similar or different from stiffness of cells expressing less or not MCSP proteins and located on rete-ridges **(Figure 3).**

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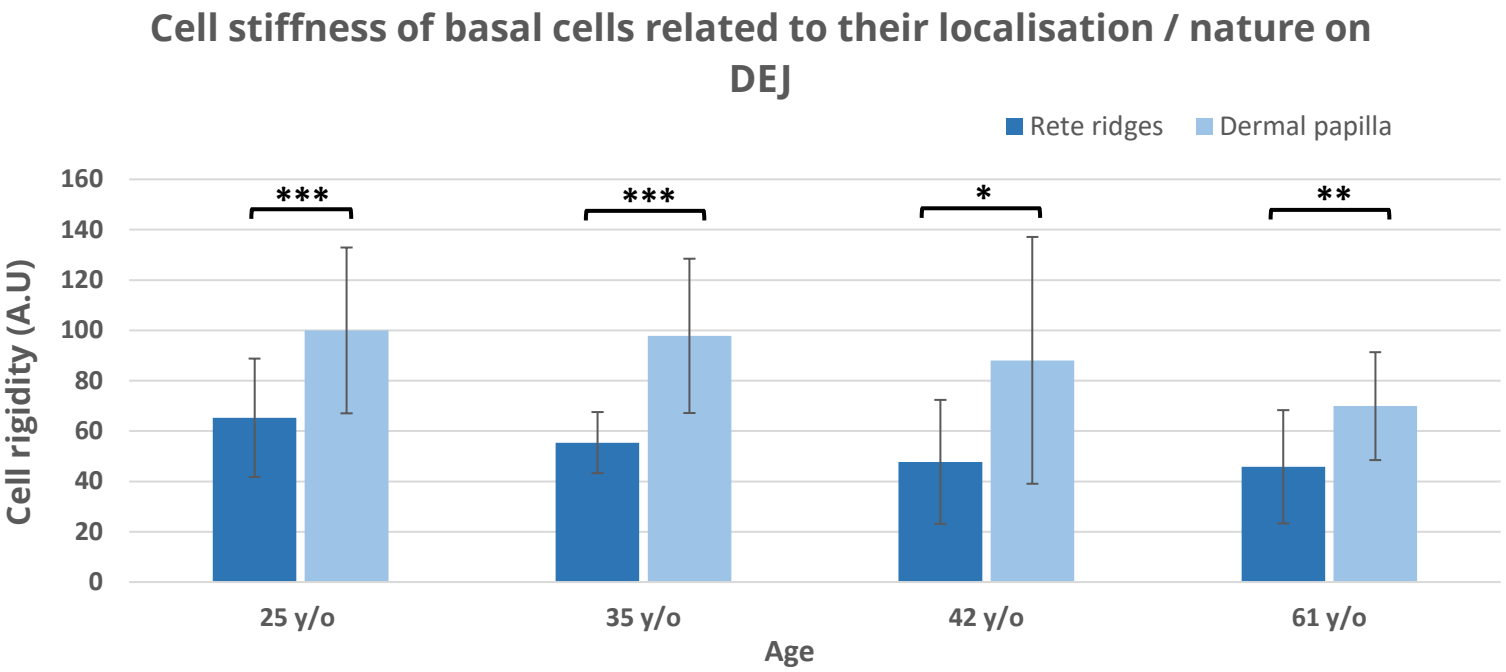

Figure 3: *Relative quantification of cell stiffness of putative interfollicular stem cells and cells located on rete-ridges. Histograms with standard deviation, represent average of cell stiffness at different ages. P-value *<0.05;**<0.005;***<0.0005.*

We have demonstrated by Atomic Force Microscopy that cells located on dermal papillae present a higher stiffness than cells located on rete-ridges. This stiffness profile is conserved but attenuates during aging, due in part to the depletion in the pool of stem cells. This AFM-based approach is reliable, fast, reproducible and allows screening of large areas to collect the largest number of samples and data.

Hence, these results indicate that a specific mechanical signature of putative interfollicular stem cells exists compared to other cell subpopulations.

Readout

Quantitative readouts are extracted from those data and images:

- Identification of distinct epidermal cell subpopulations by specific new fluorescence-based approach.
- Mechanical properties of specific pool of basal cells involved in epidermal renewal and regeneration.

Aging induces many changes in skin, being either tissular (morphological) or cellular (molecular). These changes are accompanied by alterations of skin renewal, wound healing and more generally by an alteration of skin homeostasis. This homeostasis is in part assured by basal cells and more precisely by putative interfollicular stem cells that proliferate and differentiate in all cellular types of epidermis. However, this pool of stem cells attenuates during aging.

Thus, thwarting aging by promoting the maintenance of this pool of putative interfollicular stem cells, is critical to conserve skin integrity over time.

Here, we have demonstrated by atomic force microscopy that putative interfollicular stem cells located on dermal papillae possess specific cell stiffness that differs from the one of cells located on rete-ridges.

Hence, by this approach, it is possible to evaluate and characterize the anti-aging and regenerating effects of an active ingredient aiming at maintaining skin homeostasis over aging.

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