Epidermis basal cells possess specific mechanical properties, altered during aging.

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Abstract:

Background: Basal layer of the human epidermis is composed of many cell subpopulations, including putative stem cells, involved in skin renewal. These cells are located along the undulating of the dermal-epidermal junction (DEJ) which flattens out during aging, concomitantly with a decrease of skin renewing. Specific physiological environments («niche») have been shown to be crucial for the maintenance and fate control of specific cell types.

Objectives: Our objective was to identify whether a specific mechanical environment was present on putative interfollicular stem cells and if it was altered during aging.

Methods: To assess it, we identified putative interfollicular stem cells from skin explant by an immunofluorescence-based approach and extracted their mechanical properties by Atomic Force Microscopy (AFM).

Results: We both show here that putative stem cells on dermal papilla possess a cell stiffness greater than other cells located above rete ridge. In addition, these mechanical properties are altered during aging.

Conclusions: Hence, our results demonstrate the existence of a specific mechanical signature surrounding stem cells of epidermis basal layer that increase during aging where skin renewal is altered. These results bring new insights about cell fate and cell maintenance, both involved in epidermis renewing.

Keywords: Skin aging; Interfollicular stem cell; Basal layer; Atomic force microscopy; Mechanical properties; Stiffness.

Introduction.

The skin is the largest organ of the human body and represents the body's first barrier against external aggressions. The skin is made up of 3 tissues including the dermis and the epidermis. At the interface between the dermis and the epidermis is the dermal-epidermal junction (DEJ)[1]. This last is composed of epidermal rete-ridge and dermal papilla forming undulations [2]. The epidermis is a multi-layered epithelium composed of 4 cell layers of keratinocytes in different states of differentiation. The basal layer, which lines the DEJ, is made up of different cell subpopulations [3], [4]: cells with high proliferative potential, supposed as epidermal stem cells (SCs) called interfollicular stem cells (ISCs), transit amplifier cells (TA) with limited proliferative capacity and undifferentiated keratinocytes (Fig.1). These will migrate and differentiate to form the upper layers of the epidermis [3]. Stem cells are essential for the maintenance of tissue homeostasis, for regeneration especially during injuries and in the case of the epidermis renewal, necessary to cope with the natural process of desquamation. In Human, a subpopulation of basal cells located above dermal papilla preferentially express proteins such as Melanoma Chondroitin Sulphate Proteoglycan (MCSP), LRIG1, and β1-Integrin, expressed at high level, recognized as stem cell markers [5]–[8]. ISCs are enriched in β1-Integrin allowing them to interact with proteins from the extracellular matrix (EMC) [9]. All these elements tend to locate the interfollicular stem cells above the dermal papilla. In contrast, the K15 protein, historically described as a marker of stem cells, is mainly found in cells of the basal layer located above the epidermal rete ridge [10]. All these elements tend to favour the location of a putative ISCs population above the dermal papilla. Thus, no consensus has been reached on their precise characterization.

SCs are grouped in niches, which form a specific environment that regulates the maintenance of the niche and the differentiation state of the SCs [11]. ECM proteins, such as Laminin 332, collagen IV play an important role in the regulation and the maintenance of stem cells niches [9], [11]. For example, Laminin-332, a ligand of β1-Integrin at the surface of ISCs, by is interaction with β1-Integrin, plays an important role for the regulation of epidermal homeostasis, by influencing the balance between stem cell renewal and cell differentiation [9], [12]. Mechanical environment can have an impact on cell fate, as it is the case for cancer cells. Indeed, it was described that cancer cells are able to acquire the ability to migrate, to form metastasis, through a specific mechanical environment. In addition, this acquired invasion depends on the topography and protein composition of the substrate where the cells are located. [13]. It also has been shown that the microenvironment of multipotent stem cells of central nervous system stiffens with age, and that this mechanical change is sufficient to cause age-related loss of function of this cells [14]. It has been demonstrated that the rigidity of the cell cytoskeleton can indicate a state of differentiation or a specific cell fate. [15], [16]. Hence, stem cells niches could have specific mechanical properties maintaining their stemness potential.

Skin aging is characterized by numerous modifications and alterations. Indeed, skin aging is accompanied by a reduction of the epidermal renewal rate, an alteration in the healing process and a reduction of the epidermis thickness. Moreover, with aging, the DEJ flattens and the protein composition evolves. Indeed, a decrease in the expression of Laminin-332, β4 integrin, collagen IV, VII, XVII and XVIII and Perlecan was detected during aging [17]–[20]. Finally, during skin aging, a decrease in the pool of the ISCs pool is observed and represented by a decrease in the expression of β1-Integrin and MSCP proteins by basal layer cells [5]. Our

hypothesis is that there are distinct mechanical properties between basal cells located along the DEJ either above the dermal papilla, putative ISCs, or other basal cells in epidermal rete ridge. In addition, we postulated that skin alterations observed during aging, could be due to the mechanical environment evolvement. Our approach consists in assessing the mechanical properties of basal cells by Atomic Force Microscopy (AFM). This technic enables to measure and quantify the stiffness of cells, tissues, or substrate. Our study reveals differences in the mechanical properties of basal layer cells. Actually, putative ISCs located above dermal papilla shows higher stiffness than other basal layer cells. Moreover, this mechanical environment of putative ISCs evolves with aging and tends to stiffer. Skin aging tends to homogenize the difference in cellular stiffness of ISCs with other basal layer cells of the epidermis.

Materials and Methods.

Skin explants:

Skin explants were obtained from abdominoplasties of women from 22, 36, 37, 47, 61, 64 yo (obtained from cells bank– Biopredic International). Skins were received 48 h after surgery and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with antibiotic cocktail. Serological tests for HIV, HCV and HBV were tested negative. Hypodermis was removed from the tissues by hand, with scissors and scalpel. Skin was then cut into 1 cm round pieces before freezing using liquid nitrogen and then stored at -80 °C. Frozen skin samples were embedded in OCT and cryosection of 16 µm were performed with Cryostat (LEICA CM3050S) at -20°C. Sections obtained were adsorbed on SuperFrost Plus slides (Fisher) and stored at -20 °C.

Immunofluorescence assay:

For immunostaining, cryosections were unfroze and rehydrated 10 min in 1X PBS. Sections were fixed for 20 minutes with 4% formaldehyde (Sigma) diluted in 1X PBS. After 3 rinses with 1X PBS, sections were saturated with 5% BSA (Sigma) 1X PBS, for 2 h at RT. Sections were incubated with selected primary antibodies diluted in 5% Bovine Serum Albumin (BSA) overnight at 4°C (O/N). Primary antibodies included: Goat anti-Collagen IV (dilution : 1: 100 (Sigma_AB769)), Mouse anti-MCSP (NG2) (dilution : 1: 100 (Santa cruz_SCs-80003)), Mouse anti-Collagen VII (Santa cruz_SCs-33710). Sections were washed three times in 1X PBS and incubated with secondary antibodies for 2 hours, at RT. Secondary antibodies diluted in 5% BSA included: anti-goat CF555 (dilution : 1: 800 (Sigma_Sab4600072)), anti-mouse Alexa Fluor488 (dilution : 1: 800 (Thermofisher_A28175)), anti-mouse IgG2a Alexa Fluor 555 (dilution : 1:200 (Invitrogen A-21137)), anti-mouse IgG1 Alexa Fluor 488 (dilution : 1:200 (Invitrogen A-21121)). Nuclei were stained with DAPI (dilution : 1µg/mL (Sigma)) for 20 minutes at RT. At least sections were finally washed three times in 1X PBS and stored in 1X PBS at 4°C. Mounting was done with 1X PBS-50% Glycerol (Euromedex) as mounting medium, between slide and coverslip, sealed with nail varnish and stored at 4°C.

Image acquisition and analysis:

Fluorescence imaging was performed using confocal microscopy ZEISS LSM880 (CIQLE– Centre d'Imagerie Quantitative Lyon Est, Lyon 8) with a 40× objective 1,3 Oil planApo. Images were analysed with Image J software.

Atomic Force Microscopy AFM measurements:

AFM measurements were performed with a Resolve Bioscope (Bruker Nano Surface, Santa Barbara, CA) mounted on an inverted optical DMI8 (Leica). AFM measurements were performed using a conical tip located on a flexible cantilever with a 0.35 N/m spring constant (DNP-10A, Bruker AFM probes). Before each experiment, the deflexion sensitivity of the cantilever was calibrated, and spring constant was measured using the thermal tune method. All experiments were made on epidermis sections immersed in medium PBS 1X (Capricorn-scientific) at room temperature and the standard cantilever holder for operation in liquid was used. Data acquisition was made with the Nanoscope software, 9.1.R.3 version, on AFM QNM mode, in fluid condition. Experiments consisted in acquiring force curves taken on different basal layer cells. To perform AFM measurements, we precisely localized the AFM tip at the level of the MCSP-positive cells and putative ISCs above papilla. AFM measurements were performed on collected samples from abdominoplasty of women from different ages: 22 yo

(N=1) donor; a group of 36 yo (N=1) and 37 yo (N=1); 47 yo (N=1); a group of 61 yo (N=2) and 64 yo (N=1).

AFM data processing:
Elastic modulus from skin section measurements were extracted from force curves by using the Sneddon mathematical model which implied a rigid cone indenting a flat surface:

$$F = \frac{2}{\pi}\frac{E}{(1-\upsilon^2)}\tan(\alpha)\delta^2$$

Where **α** is the half-angle of the indenter (18°), **F** is the force (from force curve), **E** is the Young's modulus, **ν** is the Poisson's ratio, and **δ** is the indentation.

Statistical analysis:
All statistical analysis was carried out using R software (R_Studio).

Results:

Identification of putative interfollicular stem cells by immunofluorescence assay

Basal layer of the epidermis is made up of different cell populations [11] including undifferentiated keratinocytes, transit amplifying cells (TA) as well as cells with high regenerative potential called stem cells (ISCs) [11]. In order to determine if the putative ISCs have specific mechanical properties, we had to discriminate them with other cell populations. To do so, an immunofluorescence-based approach was used to localize the ISCs along the basal layer, in human skin explants. For that, an MCSP labelling was used. MCSP is a cell surface proteoglycan playing a role in the spread, migration and invasion of melanoma cells, identified as a putative marker of ISCs [5], [6] due to its ability to modulate cell adhesion. MCSP labelling revealed a specific location, in pools of basal cells above the dermal papilla, in human skin at all ages tested (Fig.2). These results are consistent with what is observed in the literature [6]. Col VII labelling, a marker of the DEJ, allowed us to visualize DEJ. In addition, the Col VII stain reveals that the DEJ of a young donor has more undulations compared to the DEJ of an older donor, which is a characteristic of skin aging (data not shown).

Mechanical properties of putative interfollicular stem cells

In order to determine if putative ISCs possess specific mechanical properties, we used an Atomic Force Microscopy (AFM) based approach to analyse and extract mechanical properties of these cells. As regard as mechanical characteristics, we were mainly interested in cell stiffness properties, but other parameters can be analysed such as viscoelasticity or cell adhesion. Firstly, we measured cellular stiffness of basal layer cells. The cellular stiffness takes account for several elements such as intracellular organelles, cytoskeleton etc. and extracellular proteins. This approach allows to detect specific mechanical environment present on different cell subpopulations in the basal layer. We have therefore carried out AFM measurements specifically at the level of putative ISCs above the papilla and at the level of the basal cells above rete ridge (Fig.3).

In the skin from a 22 yo donor (Fig.4A), we identified a difference in cell stiffness depending on cell population. Indeed, the average cell stiffness of ISCs above dermal papilla is 1.39 times significantly higher than stiffness of basal layer cells above rete ridge. The same observation is found in a group of 36-37-years-old skins and in a skin of 47 yo (Fig.4A). Indeed, average putative ISCs stiffness is respectively 1.56 times and 1.34 times more important than stiffness in cells from rete ridge.

Thus, these results indicate that from 22 to 47 yo, some specific mechanical properties exist between cell subpopulations. Indeed, cell stiffness of putative ISCs is higher than basal layer cells located above rete ridge.

Skin aging alters the mechanical properties of putative interfollicular stem cells

We next wanted to identify if skin aging could alter mechanical properties of cells from the basal layer. We therefore compared the average cellular stiffness of basal layer cells depending

on their location during aging. Results revealed that for a group of 61-64-years-old skin, the average cell stiffness of putative ISCs is similar of the one observed for basal layer cells above rete ridge (Fig.4A). Indeed, no significant differences about cellular stiffness were detected between cells from the basal layer. Moreover, we observed that the average stiffness of putative ISCs significantly increases during aging (Fig.4B). Indeed, cell stiffness switches from 57 A.U in a 22 yo skin to 135 A.U in a group of 61-64 yo skin, either a significant increase by a factor of 2.36. At last, we also detected that the average cell stiffness of basal layer cells above rete ridge also significantly increases during aging (Fig.4C). These data indicate that the cellular stiffness of cells from the basal layer increases with aging. Thus, our results reveal that during aging, there is an alteration of the mechanical properties of the cells from the basal layer of the epidermis. Indeed, putative ISCs seems to lose their specific mechanical property with aging and a global increase of cellular stiffness of the basal layer of epidermis increases with aging.

Attenuation of differences in mechanical properties of basal cells during skin aging

During skin aging, it is described that the pool of interfollicular stem cells decreases [5]. It has been demonstrated that the rigidity of the cell cytoskeleton can indicate a state of differentiation or a specific cell fate. [15]. We sought to determine whether there could be a correlation between the decrease in the putative ISCs pool during aging and a change in the mechanical properties of the putative ISCs cellular environment. We have therefore analysed the ratio of cell stiffness between putative ISCs with basal layer cells above rete ridge. We observed that the ratio of cell stiffness decreases during aging (Fig.4D). Indeed, for the group of 36-37yo skins, the ratio of stiffness is equal of 1.56. In contrast this ratio in the group of 61-64 yo skins falls to 1.03. A ratio close to 1 indicates that mechanical characteristics between the two subpopulations are similar and so could indicate a similar state of cell fate. Based on this hypothesis, our results indicate that cell subpopulations tend to slightly homogenize during aging. At last, our results show that during aging, the decrease in the putative ISCs pool correlates with the modification in the mechanical properties of putative ISCs environment.

Discussion.

Basal layer of the epidermis is composed of different cell subpopulations including cells with a high regenerative potential called putative ISCs. In human, despite numerous studies, no markers have been specifically associated to epidermis ISCs, unlike in mice, meaning that no consensus have been established about the precise location of epidermal stem cells. In this study we were interested in analysing mechanical properties of different cell subpopulations environment of epidermis basal layer. Indeed, the interest for the analysis and the effect of the mechanical properties at the cellular and tissue level is growing, bringing many complementary information which are not accessible by the traditional imagery techniques, or protein expression. It is described that the mechanical environment of the cells, influences cell fate [21]. We know that the basal layer of the epidermis contains heterogeneous cell subpopulations and especially some cells with a strong regenerative potential involved in skin renewal. During aging, the DEJ, on which the basal cells lie, flattens, inducing a modification of the environment of basal cells. Our hypothesis was that distinct mechanical properties exist between cells located above the dermal papilla, i.e. putative ISCs that asymmetrically expressed specific proteins, compared to other basal cells in epidermal rete ridge. In addition, we make the postulate that this mechanical environment could evolve during aging and could explain or correlate with skin alterations observed during aging.

We first performed an immunofluorescence-based approach to discriminate putative ISCs at the basal layer in frozen sections of skins from women donors. The aim being to use their location to precisely measure the mechanical properties of cell subpopulations by AFM. Based on the literature, we used MCSP proteins described to be a marker of putative ISCs [6]. Indeed, MCSP is expressed by keratinocytes expressing high level of β1-Integrin in cells from dermal papilla and β1-Integrin is identified as an ISCs marker due to its role in modulating cell adhesion. All basal layer cells express β1-Integrin, but a specific accumulation of the protein is detected in cells located above dermal papilla [9]. Indeed, β1-Integrin is identified as an important regulator of epidermal homeostasis, influencing the balance between stem cell renewal and differentiation [9]. It has been shown that basal layer cells expressing high level of β1-Integrin also express the MCSP protein [5], [6]. Once isolated, these MCSP positive cells are able to form self-renewal clones, indicating their potential in controlling SCs lineage. The location of MCSP positive cells allowed us to highlight a subpopulation of cells, putative ISCs, specifically located above the dermal papilla. Moreover, with aging, skin explants have loss the DEJ undulation and tend to loss the expression of MCSP protein. This confirms the aging state of the skin, since skin aging is characterized by a decrease in the undulation of the DEJ and a loss of the putative ISCs pool [5], [20].

To determine if a specific mechanical environment surrounding cell subpopulations exist, we then performed a relative analysis of the mechanical properties of basal cells by AFM. For this purpose, we targeted cells located at the top and at the lowest place of the undulations of the DEJ, that express or not MCSP protein. First, we demonstrated that putative ISCs, located above dermal papilla, possess a higher cell stiffness than basal layer cells above rete ridge. This difference in cell stiffness is maintained on skin samples from 22, 36, 37 and 47 yo donors.

Cellular stiffness depends on different parameters such as the stiffness of the cytoskeleton, cellular organelles or such as cell-cell junctions (desmosome, intermediate junctions), cells-basal membrane junctions (hemidesmosome). This difference in cell stiffness between the putative ISCs and the basal layer cells above rete ridge could be explained by the presence of a specific cellular environment. Indeed, epidermal ISCs are grouped in niches, forming a specific environment, where cell-cell and cell-basal membrane junctions, with ECM protein such as Laminin-332, are very important. These interactions can regulate the maintenance of the niche and the differentiation of epidermal ISCs [11], [12]. The difference in stiffness properties of basal layer cells can also be explained by the location of putative ISCs with respect to the DEJ. Indeed, ISCs are systematically located above the papilla, so it is possible that the topography of the papilla, different from the one of the rete ridge, influences the mechanical properties of the cells. It has been described, in an *in vitro* study, that the topography of the substrate can affect the behaviour of epidermal ISCs and could promote or inhibit their differentiation [22]. Moreover, it was shown that the patterning of epidermal ISCs depend on the mechanical forces exerted at the intercellular junctions in response to the undulations (rete ridge and dermal papilla) of the DEJ [23]. This study differs from a biological model since they use an *in* vitro approach for culturing primary human keratinocytes on an elastomer substrate. Contrarily to what we observed *ex vivo*, they revealed a higher cell stiffness for rete ridge cells than papilla located cells.

Thereafter, we have shown that aging has an impact on the mechanical properties of skin. Indeed, putative interfollicular ISCs seems to lose their specific mechanical property with aging. Moreover, stiffness of all basal layer cells increases during aging. Finally, the ratio of cell stiffness of putative ISCs/cells above rete ridge is smaller at 61-64 yo than in at 36-37 yo. A ratio close to 1 indicates that mechanical characteristics between the two subpopulations are similar and so could indicate a similar state of cell fate. It was shown that during aging, the pool of putative ISCs, within the niches, decreases, due to a decline in putative ISCs self-renewal [5]. It is therefore possible that the decline of the putative ISCs pool, during aging, tends to homogenize the cellular stiffness of the basal cells of the epidermis.

Moreover, in aged donors, the niche environment is modified and is less favourable to maintain a pool of cells with generative potential, thus altering cell-cell and ECM-cell interactions and thus can leading to a modification in the cellular stiffness. Otherwise, during aging, it has also been demonstrated that the DEJ loses its undulations, thus becoming flat [20]. It is accepted that mechanical signals from cells are translated and transmitted directly to the nucleus, modulating gene expression and thus cell fate [21], [22]. This is the case for cancer cells, where a mechanical signal influences their behaviour and promotes their migration [13]. It has been shown that the microenvironment of multipotent stem cells of central nervous system stiffens with age, and that this mechanical change is sufficient to cause age-related loss of function of this cells [14]. It is therefore possible that these changes in DEJ topography during aging modify the native mechanical environment of basal cells and hence modify cell fate promoting putative ISCs differentiation against cell proliferation. This hypothesis could explain the loss of the stem cell pool during aging. These results therefore suggest that it exist a correlation between the presence of undulation of the DEJ and the persistence of a putative ISCs pool. Moreover,

whatever the hypothesis, it would seem that there is a link or a correlation between the existence of putative ISCs and the mechanical properties of the skin.

Our results have therefore allowed us to demonstrate the existence of a specific mechanical signature of putative ISCs in the basal layer of the skin. Thus, a model of skin aging based on the mechanical properties of these putative ISCs seems likely. The presence of undulations within the DEJ could influence the mechanical properties of the putative ISCs and therefore their functions and cell fate. Hence, It would be interesting to study the mechanical properties of the DEJ, its size, shape, especially at the areas of rete ridge and papilla, and determine if aging that alters its protein composition also alters its mechanical properties [19]. Knowing that cell rigidity depends on forces exerted on cell-cell and cell-membrane basement junctions, the analysis of junction stiffness could bring more knowledge about the higher stiffness observed for ISCs. More generally, it would be necessary to increase the number of measurements on different donors to confirm these results and to be able to propose a robust model of skin aging based on mechanical properties of these basal cells.

In addition to a better understanding, these results bring new insights in the characterization of active ingredient with anti-aging effect, targeting skin renewal or skin regeneration after wound, directly involving basal cells and putative ISCs of epidermis.

Conclusion.

Our results highlight the existence of a specific mechanical signature of putative interfollicular SCs, specifically located above the dermal papilla (Fig.5). We show that putative ISCs have a higher cellular stiffness than basal cells located above the epidermal rete ridge. This difference in stiffness seems to don't persist in old skin. On the other hand, an increase in the rigidity of basal cells of the epidermis is observed during aging. Thus, it seems that during aging, the mechanical properties of the cells from the basal layer tend to homogenize. This correlates with hallmarks of skin aging where a decrease in the pool putative interfollicular SCs and in skin turnover is observed.

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Conflict of Interest Statement.

The authors declare no competing interests.

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Figure legends.

Fig1:
Schematic representation of human skin with the epidermis, dermal-epidermal junction (DEJ) and the dermis. Epidermis is made of different cell layer including the stratum basal (basal layer). Basal layer is made up of transit amplifier cells (TA), undifferentiated keratinocytes and interfollicular stem cells. DEJ is wavy and has dermal papillas and epidermal rete-ridges. Interfollicular stem cells (ISCs) are grouped in a niche above the dermal papilla and highly express the MSCP protein as well as β1-Integrin.

Fig2:
Localisation of interfollicular stem cells of epidermis in skin tissue section from 36 yo donor, observed with a confocal microscope, stained for Col VII (green), MCSP (red), DAPI (blue). Scale bar = 20 µm.

Fig3:
(A) Epidermis skin sections from 36 yo donor, observed with a confocal microscope, stained for Col VII (green), MCSP (red), DAPI (blue). The orange triangles represent the areas of measurement AFM (1) at the level of the basal cells above the rete ridge (2) at the level of the ISCs above the dermal papilla. (B) Brightfield acquisition of a section of human abdominal skin (22 yo) showing the dermis and the epidermis. The white dotted line represents the dermal epidermal junction. Small squares indicate positions of AFM measurements of 25x25 µm at the dermal papilla and rete ridge. Scale bar = 25 µm.

Fig4:
Analysis of average cell stiffness by AFM on epidermis frozen sections from human skins of different ages: 22 yo (N=1) donor; a group of 36 yo (N=1) and 37 yo (N=1); 47 yo (N=1); a group of 61 yo (N=2) and 64 yo (N=1) (n = 3 sections for each donor and n>20 AFM measurements for each donors). For AFM measurements, 3 independent areas by sections for each donor with average 3 AFM measurements of 25 µm*25 µm size at the level of interfollicular stem cells (ISCs) and at the level of cells above rete ridge (RR). (**A**) Histogram representing average cell stiffness of ISCs above dermal papilla and basal layer cells above rete ridge (RR) depending on the age of the donor. **(B-C)** Evolution of the average cell stiffness (A.U) of interfollicular stem cells (ISCs) (**B**) or basal layer cells above rete ridge (RR) (**C**), during aging. (**D**) Evolution of the average cell stiffness (A.U) of interfollicular stem cells (ISCs), in orange, and of basal layer cells above rete ridge (RR), in blue, during aging. The values on the graph represent the ratio between the average cell stiffness of the ISCs and the basal cells above the rete ridge. ns = not significant; *, p-value < 0.05; ***, p-value < 0.0005; ****, p-value < 0.00005.

Fig5 :
Schematic representation of the observed average cellular rigidity of basal cells in young skin and old skin. +++ indicates very high cellular stiffness; ++ moderately high cellular stiffness; +- average cellular stiffness.

Figures.

Fig.1

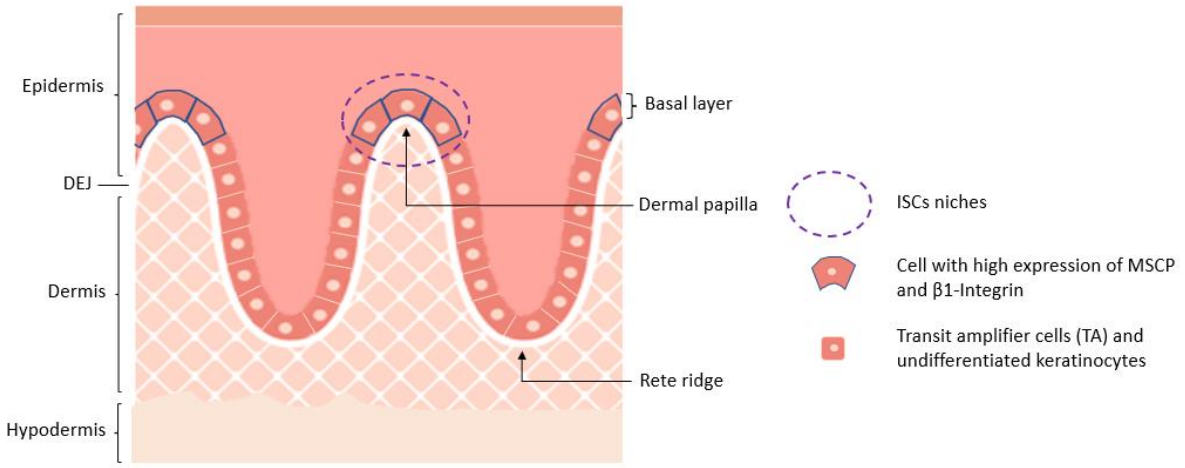


Fig.2

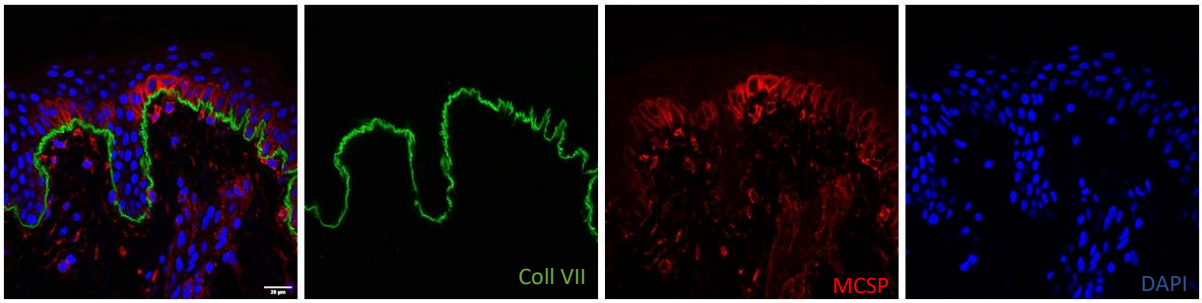
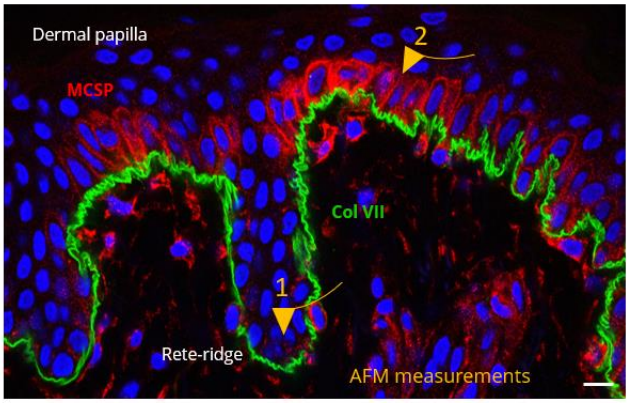


Fig.3

A



B

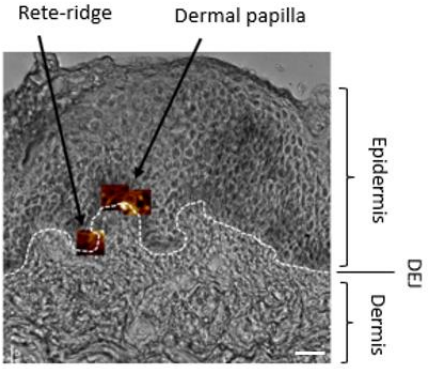
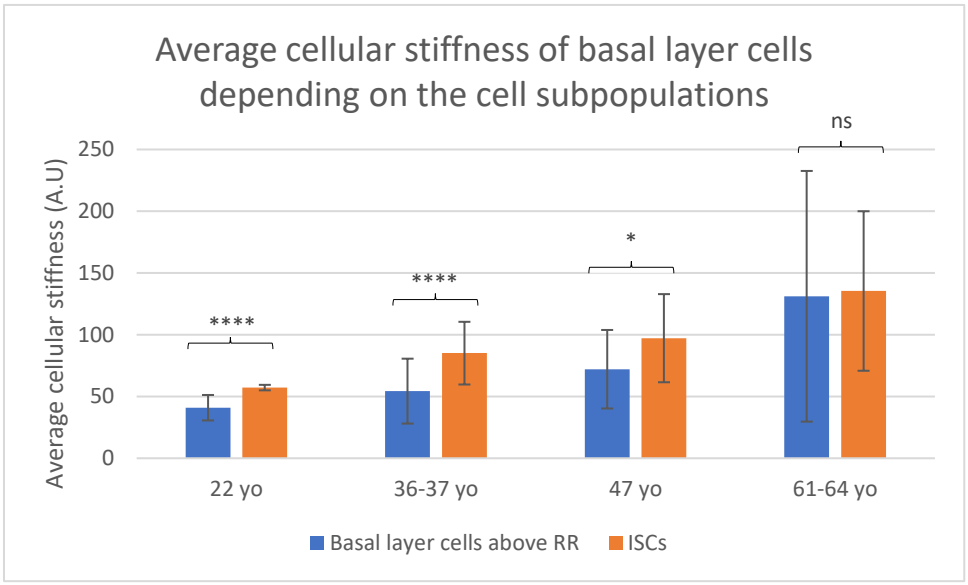
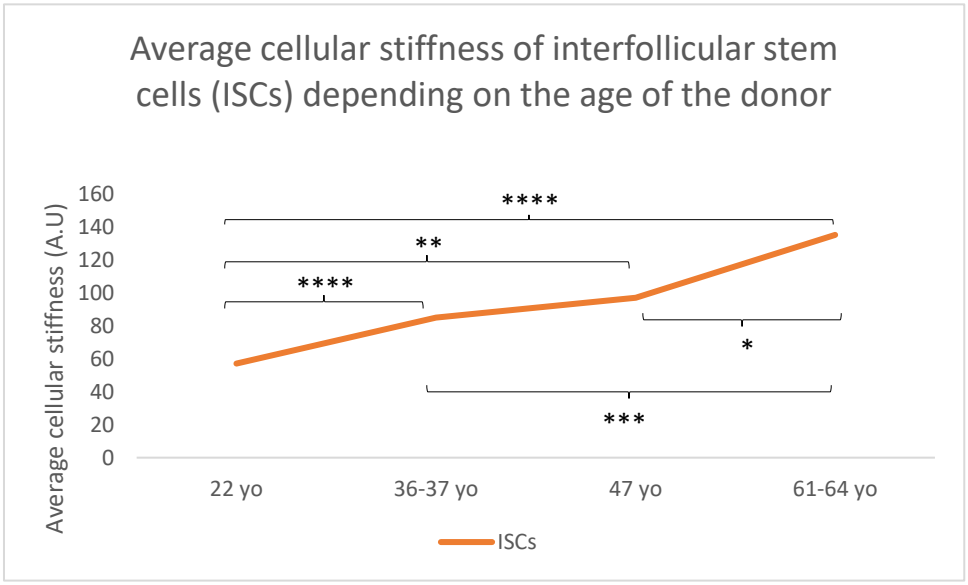


Fig.4

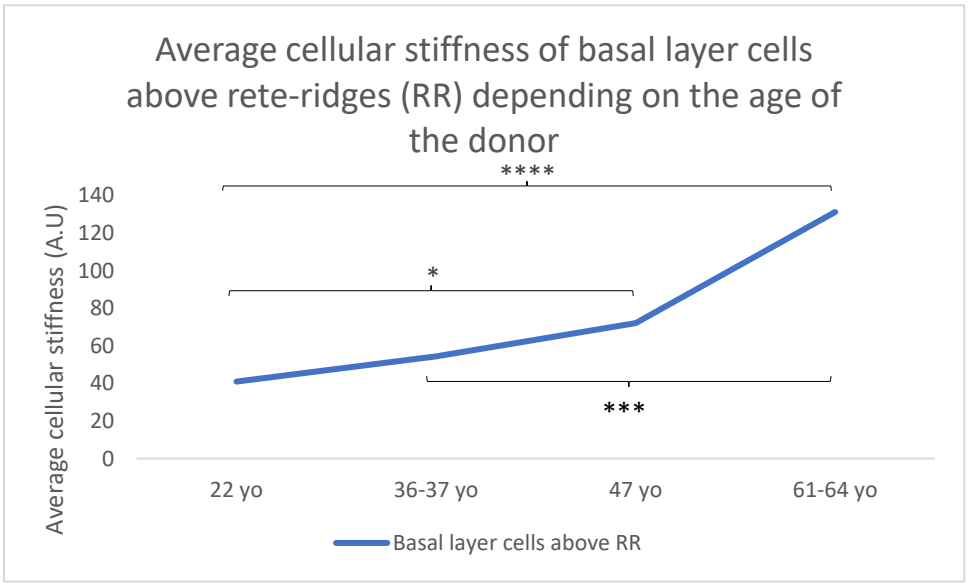
A


Average cellular stiffness of basal layer cells depending on the cell subpopulations
Average cellular stiffness (A.U)
250
200
150
100
50
0
****
****
*
ns
22 yo
36-37 yo
47 yo
61-64 yo
Basal layer cells above RR
ISCs

B


Average cellular stiffness of interfollicular stem cells (ISCs) depending on the age of the donor
Average cellular stiffness (A.U)
160
140
120
100
80
60
40
20
0
****
**
****
*
***
22 yo
36-37 yo
47 yo
61-64 yo
ISCs

C


Average cellular stiffness of basal layer cells above rete-ridges (RR) depending on the age of the donor
Average cellular stiffness (A.U)
140
120
100
80
60
40
20
0
****
*
***
22 yo
36-37 yo
47 yo
61-64 yo
Basal layer cells above RR

D

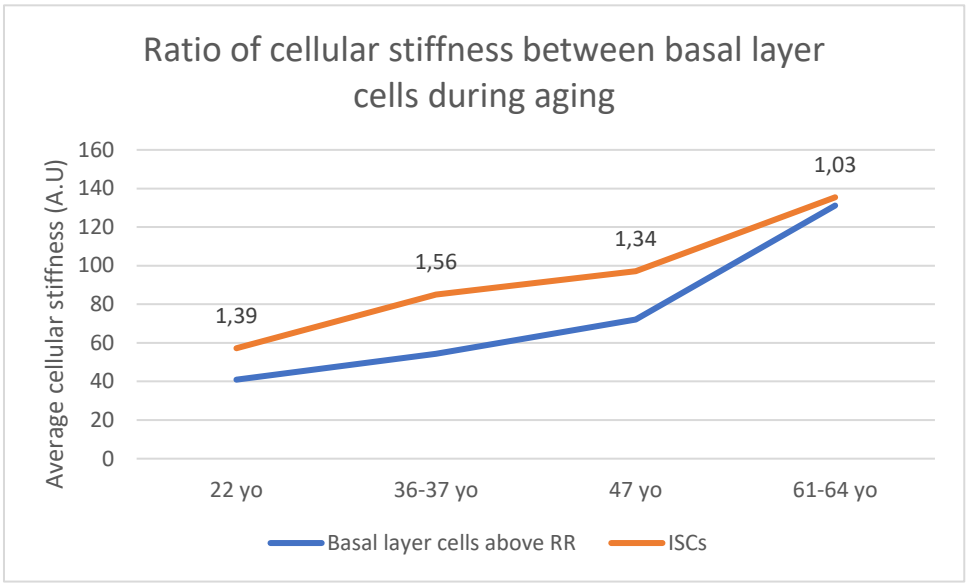
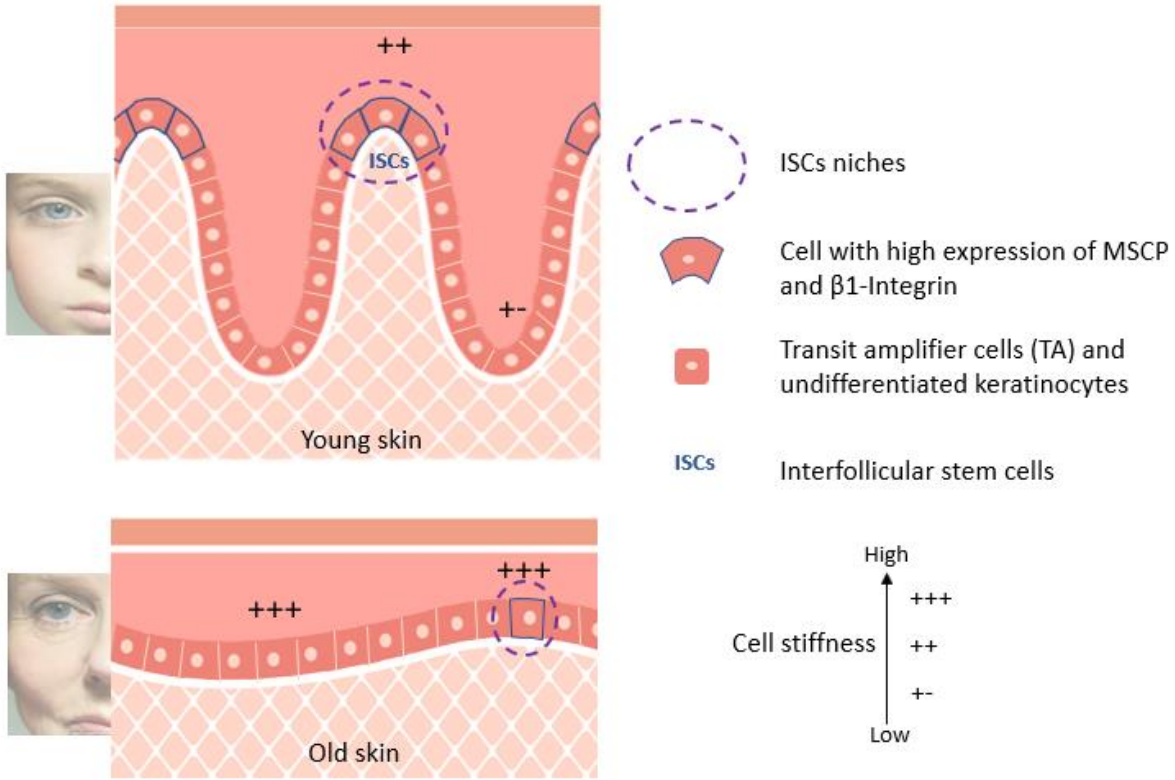

Ratio of cellular stiffness between basal layer cells during aging
Average cellular stiffness (A.U)
160
140
120
100
80
60
40
20
0
1,39
1,56
1,34
1,03
22 yo
36-37 yo
47 yo
61-64 yo
Basal layer cells above RR
ISCs

Fig.5


++
ISCs
+-
Young skin
+++
+++
Old skin
ISCs niches
Cell with high expression of MSCP and β1-Integrin
Transit amplifier cells (TA) and undifferentiated keratinocytes
ISCs
Interfollicular stem cells
High
Cell stiffness
+++
++
+-
Low