Immunocompetent and Microvascularized Human Full-Thickness skin equivalent as a new advanced 3D model for the evaluation of cosmetics active ingredients targeting skin aging

Laure, Crabbe Vert[1*]; Sandrine, Heraud[2]; Kilian, Laho[2]; Juliette, Sage[1]; Olivier, Jeanneton[1]; Jocelyne, Franchi[1]; Carine, Nizard[1]; Karl, Pays[1]; Bruno, Bavouzet[1]; Amélie, Thépot[2]; **Morgan, Dos Santos[2*]**

[1] Life Sciences Department, LVMH Recherche, France; [2] Labskin Creations, France

*Morgan Dos Santos, LabSkin Creations, 56B Quai Joseph Gillet, 69004, Lyon, France. +33 482 906 498, morgan.dossantos@labskincreations.fr

*Laure Crabbe Vert, LVMH RECHERCHE, 45804 Saint Jean de Braye, France. +33 785 579 404, lvert@research.lvmh-pc.com

Abstract (Maximum of 200 words)

As a first barrier between the external environment and the body, skin harbors a range of immune cells that initiate a rapid innate immune response against pathogens, trauma, or photoexposure. However, it remains unclear whether age-dependent changes in skin microenvironment could perturb this important response. Here, we built a functional immunocompetent and endothelialized tissue-engineered human model to unravel how aging impacts skin immune cells residence and activation in response to stress. $CD34^+$ hematopoietic progenitors from cord blood were integrated into a microvascularized skin equivalents (SEs) prepared from aged or young donors. Upon UV irradiation, immune Langerhans cells (LCs - CD207/Langerin) and dermal dendritic cells (DDC - CD209/DC-sign-positive) respectively found in the epidermis and the dermis of young SEs, were mobilized within the tissue to promote the proper immune response. They notably reached the capillary-like ring structures embedded in the neo-synthetized extracellular matrix. In contrast, we observed significant changes in the morphology of skin engineered from aged cells that impeded

immune cells microenvironment and affected their functionality. Our new immunocompetent and endothelialized SE may be a relevant tool to further decipher the impact of aging microenvironment on immune cells and identify new cosmetic active ingredients on more physiological basis.

Keywords: Aging; Immune cells; 3D skin model; UV stress; endotheliazed.

Introduction

The skin is a crucial barrier between the external environment and the body acting as the first physical and immune line of defense against external threats. This performance is achieved with a fine-tuned resident immune system within the skin. Diverse and functionally specialized subsets of immune cells populate the skin and initiate a rapid innate immune response against pathogens, trauma, or photoexposure [1]. Cells from the innate immune response include keratinocytes, the major cell type in the epidermis, endothelial cells, responsible for the formation of capillaries embedded in the dermal extracellular matrix, and Langerhans cells (LCs) and dermal dendritic cells (DDCs). Both LCs and DDCs are dendritic cells specialized in antigen presentation and abundantly found in peripheral tissues such as skin where they function as immune sentinels. LCs locate at the skin epidermal barrier where they account for approximatively 2 to 3% of epidermal skin cells [2]. Derived from myeloid progenitors, mature LCs are characterized by the expression of Langerin (CD207), a receptor involved in the presentation of non-peptide antigens during skin inflammation. They migrate to the epidermis where their long-term residency is preserved by E-cadherin-mediated adhesion to surrounding keratinocytes. The final differentiation of LC precursors depends on the cytokine environment of the epidermis [3]. Upon stimulation, LCs trigger a series of immune response by migrating from the epidermis to the lymph node through the lymphatic vessels present in the dermis [2]. Production of cytokines, particularly TNF-alpha, IL1a and IL1b facilitates migration of LCs out of the epidermis. In addition, production of matrix metalloproteases (MMPs) is upregulated in activated LCs to facilitate the passage of the cells across the basement membrane. Remarkably, it was shown that when exposed to UV irradiation, the number of LCs in the epidermis can then drop by almost 50% of the steady state value and it can take several days to be recovered

[4]. DDCs reside in the dermis and also participate in the recognition of stressors, sensing danger and initiating a proper response. They express DC209/DC-SIGN (Dendritic Cell-Specific Intercellular Adhesion molecule-3-Grabbing Non-integrin), a receptor that recognizes pathogens. Similarly to LCs, DDCs migrate upon stimulation to reach the lymph node. MMPs are essential for migration of LCs and DDCs through the dermal tissue matrix, by cleavage of collagen IV [5].

The skin microenvironment plays a major role in this innate response, firstly to reach a full differentiation and residence of both LCs and DDCs, secondly for the cells to be properly activated and gain their migratory phenotype, thirdly to locally adapt the extracellular matrix structure to pave the way through the tissue to the microvasculature. For this reason, we asked whether age-dependent skin structural changes could compromise immune cell microenvironment and affect their functionality. To date, this important question could not be properly tackled due to the lack of a relevant *in vitro* 3D immunocompetent and microvascularized SE model.

Here, we aimed to unravel how skin aging impacts skin immune cells response to stress. We developed for the first time a functional human full-thickness skin equivalent (SE) comprising both cutaneous immune and microvascular components to study the impact of both intrinsic and extrinsic aging. Altogether, our results strongly suggest the view of aging as a key driver of skin immune response in a complex 3D skin model. Our new immunocompetent and endothelialized SE may be a relevant tool to further decipher the impact of aging microenvironment on immune cells and identify new cosmetic active ingredients on more physiological basis.

Materials and Methods

Ethical considerations and human cells isolation

This study was approved by the ethical research committee “Comité de Protection des Personnes Sud-Est II” and declared to the French Research Ministry (declaration no. DC- 2024-6232 delivered to LabSkin Creations, Lyon, France). Adult skin samples were collected according to the Declaration of Helsinki Principles. A written informed consent was obtained from skin donors according to the French bioethical law of 2014 (loi 94–954 du 29 Juillet 1994). Primary cultures of human fibroblasts, keratinocytes and endothelial blood and lymphatic cells were established from healthy skin biopsies obtained from infant (> 5 years old) and adult donor (57 years old). Normal

human epidermal keratinocytes (NHEK), dermal fibroblasts (NHDF), dermal microvaslurized cells (HDMVEC) were isolated from foreskin and human abdominal skin.

Cord blood CD34$^+$ cell culture

Isolated CD34$^+$ progenitors were cultured for 6 days in RPMI, supplemented with 10% heat inactivated fetal calf serum and 100 U/ml penicillin, 100 µg/ml streptomycin. Cultures were supplemented with recombinant human GM-CSF, human TNFα and TGFβ.

Full-thickness microvascularized human skin models engineering

3D full-thickness reconstructed skin model was obtained by co-culturing NHDF and HDMVEC in a scaffold made of collagen, glycosaminoglycans and chitosan (LabSkin matriX^{TM}, Lyon, France) during 21 days under optimized cell culture conditions for ECM neo-synthesis. NHEK were then seeded on the top of the dermal endothelialized equivalent constructs and raised at the air/liquid interface to allow the formation of the epidermal compartment.

Fifteen days after being raised to the air–liquid interface, skin substitutes were exposed to both UVA and UVB irradiations. Skin equivalent samples harvested 4h (UV+4H) and 24h (UV+24H) post-irradiation were immediately fixed in neutral buffered formalin 4% for 24h and embedded in paraffin or in OCT compound and frozen at −80°C, for histological and immunohistological analysis, respectively. For each cell culture condition and analysis, 3D skin equivalents were produced in triplicate.

Integration of Langerhans cell/dendritic cell precursors

To obtain the integration of Langerhans cells/dermal dendritic cells into the endothelialized skin equivalent CD34$^+$ progenitors were integrated 3 days after keratinocyte seeding.

Histological staining

Full-thickness human 3D endotheliazed and immunocompetent and endotheliazed skin equivalent were fixed in formalin and embedded in paraffin. 5-µm paraffin-embedded sections were deparaffinized and stained with Haematoxylin-Phloxin-Saffron (HPS) to evaluate the global

cutaneous structure of samples. The images were taken with the system optical microscope Axio-Observer D1/high resolution camera Axiocam (Zeiss). Image processing and analysis were performed using the software Image J.

Immunohistological analysis

For immunofluorescence on paraffin sections, after heat-mediated antigen retrieval treatment, tissue sections were incubated in 5% H_2O_2/3% bovine serum albumin to inactivate endogenous peroxidases. Non-specific binding was blocked in PBS containing 5% of BSA. Sections were then incubated with Langerin (CD207) or DC-SIGN/CD209 primary antibodies diluted in PBS/BSA 5% overnight at room temperature. After incubation for 1 hour with secondary AlexaFluor-488- or Alexa-568-conjugated anti-mouse or anti-rabbit antibodies (Molecular Probes, Invitrogen). Nuclear counterstaining using DAPI was carried out routinely. As a negative control, primary antibody was replaced by the corresponding IgG class.

For immunohistochemistry, after incubation for 1 hour with the peroxidase labelled polymer conjugated to secondary antibody, the antigen was detected with 3,3-diaminobenzidine tetrachloride (Dakocytomation) as the substrate. Tissue sections were subsequently counterstained using Harris' hematoxylin (25%, Sigma Aldrich).

Image acquisition and analysis

Specimens stained in HPS were observed using an Axioskop 2 Plus optical microscope (Zeiss), and images were captured using DS-Ri1 CCD camera (Nikon) and NIS-Elements software (Nikon). Sixteen-bit images were saved in an uncompressed tagged image file format (tiff). Nine representative images were captured for each condition in the same manner.

Image processing and analysis were performed using the software ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2017).

For immunohistochemical staining, positively stained-tissue areas were automatically detected and segmented from other pixels. Images were then converted in binary images, treated by

mathematical morphology and sieved for isolating the regions of interest. The surface area of interest was measured automatically. For immunofluorescent staining, images were first converted to RGB images and then deconvolved into their red, blue and green components. The red component was identified as the biological target. Depending on the biological target, positively stained-tissue areas were normalized by total dermal or epidermal area (results expressed in percentage of density) or normalized by the dermal-epidermal junction length.

Multiplex cytokines quantification in RHS

Levels of IL-6, G-CSF, Fractalkin, IP-10 and TNF-beta in RHS supernatants were quantified using the MILLIPLEX Multi-Analyte Profiling (MAP) Human Cytokine/Chemokine assay kit (HCYTOMAG-60K, Millipore) and MAGPIX Multiplexing System (Luminex Corporation, Austin, TX, USA) according to the manufacturer's instructions. Data were analyzed using xPONENT 4.2 software.

Statistical Analysis

All data are presented as mean values ± standard deviations. Statistical significance in the data was assessed running Student's t-test. Each set of data relates to a comparison versus untreated control. Statistical significant differences are indicated by asterisks as follows: *P < 0.05, **P < 0.01 and ***P < 0.001.

Results

Immunocompetent and microvascularized skin equivalents display a consistent morphology and contain vascular and lymphatic capillary-like structures.

First, we built a functional immunocompetent and endotheliazed tissue-engineered human model using $CD34^+$ hematopoietic progenitors from cord blood. They were integrated into a microvascularized skin equivalents (SEs) prepared by co-culturing dermal fibroblasts and microvascular endothelial cells into a unique porous scaffold made of chitosan-cross-linked collagen glycosaminoglycan polymer, subsequently epidermized with human keratinocytes (Figure 1). After a total of 45 days of 3D cell culture, histological analysis confirmed that the cultured SEs were morphologically consistent and presented a cohesive dermal-epidermal structure.

Keratinocytes produced a full-thickness well-organized and terminally differentiated epidermis including a multi-layered *stratum corneum*.

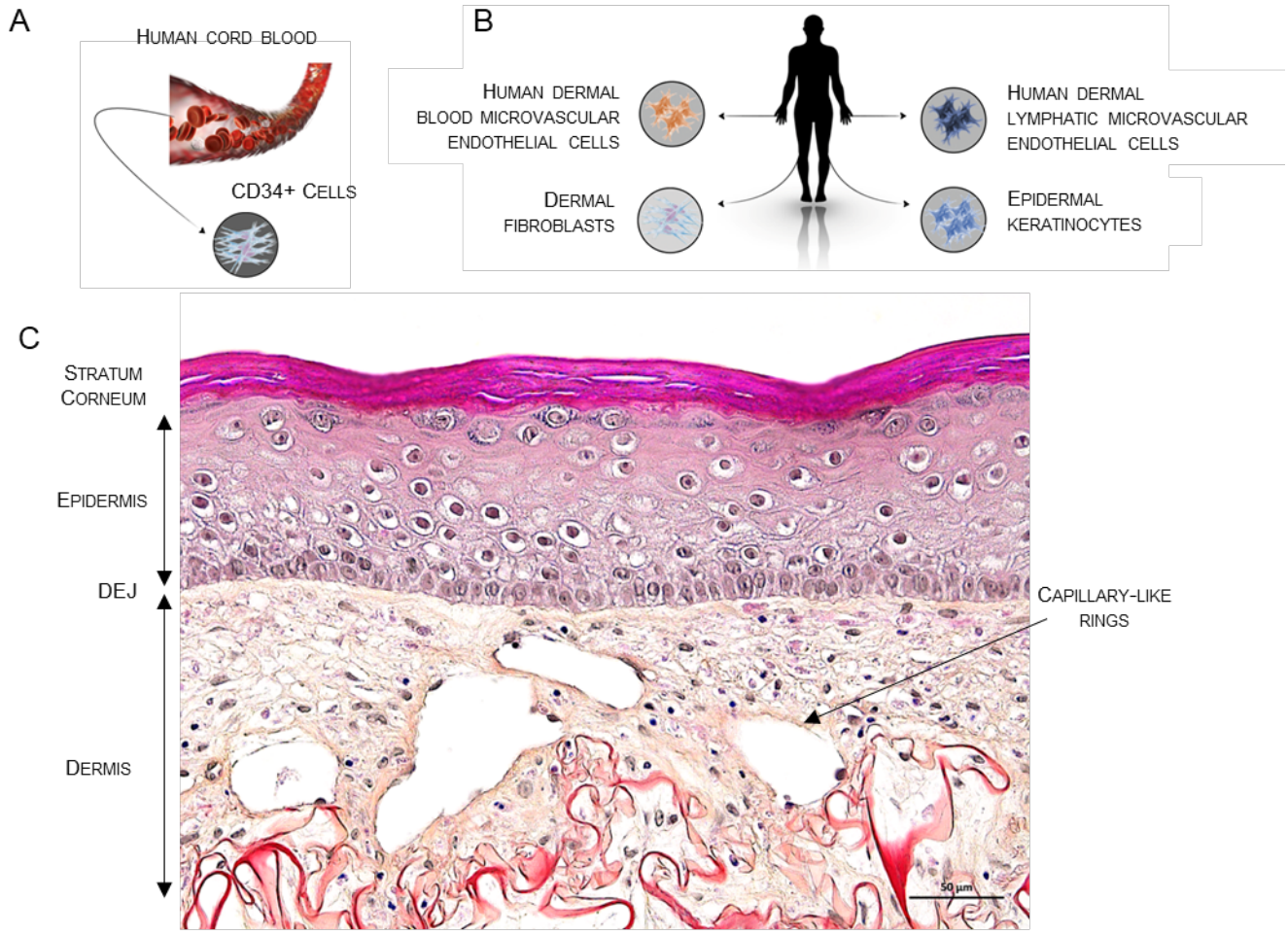


FIGURE 1: Immunocompetent and microvascularized skin equivalents display a consistent morphology
A and B- origin of the cells used to built the 3D-skin model.
C- Representative histology image of the 3D-skin model using Hematoxilin Phloxin Saffron staining.

At the dermal level, endothelial cells adhered and proliferated to form numerous capillary-like ring structures embedded in the neo-synthetized extracellular matrix. Remarkably, immunostaining experiments using vascular (CD31) and lymphatic (podoplanin) vessels markers further revealed the presence of both types of capillaries in the SEs (Figure 2).

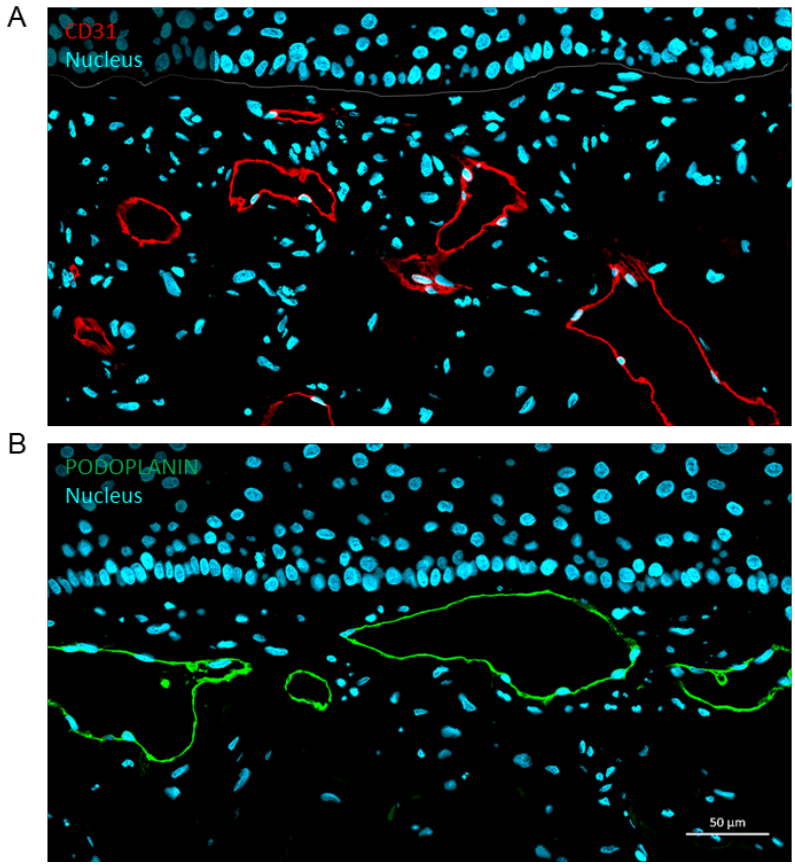


FIGURE 2: Vascular and lymphatic capillary-like structures are formed in the SEs
A-Representative immunostaining of CD31 (red) to visualize vascular capillary-like structures in the SEs. Nuclei are labelled with DAPI (cyan).
B-Representative immunostaining of podoplanin (green) to visualize lymphatic capillary-like structures in the SEs. Nuclei are labelled with DAPI (cyan).

Integration of CD34$^+$ progenitors during the reconstruction procedure results in the residence of differentiated dendritic cells.

Skin dendritic cells mostly originate from CD34$^+$ precursors that mature and differentiate with the help of the skin microenvironment. CD34$^+$ cells were therefore seeded during the reconstruction procedure, a few days after the seeding of keratinocytes on top of the dermal equivalents and before initiation of the pluri-stratification of the epidermis. At the end of the tissue reconstruction, the residence of LCs and DDCs was assessed in the SEs using langerin/CD207 staining and DC-SIGN/CD209 immunostaining, respectively (Figure 3). Langerin-positive cells were detected in the epidermis (Figure 3A), and they displayed the typical dendritic morphology of epidermal Langerhans cells. No langerin-positive cells were detected in the upper dermis. In contrast, numerous dermal CD-SIGN-positive dendritic cells were detected in the upper part of the dermal compartment (Figure 3B). Altogether these results strongly suggest that the reconstructed skin provided to proper microenvironment for CD34$^+$ precursors to efficiently differentiated into LCs in

the epidermis, and DDCs in the dermis. In addition, both types of immune cells displayed the dendritic morphology that ensure their long-term residency within the skin tissue.

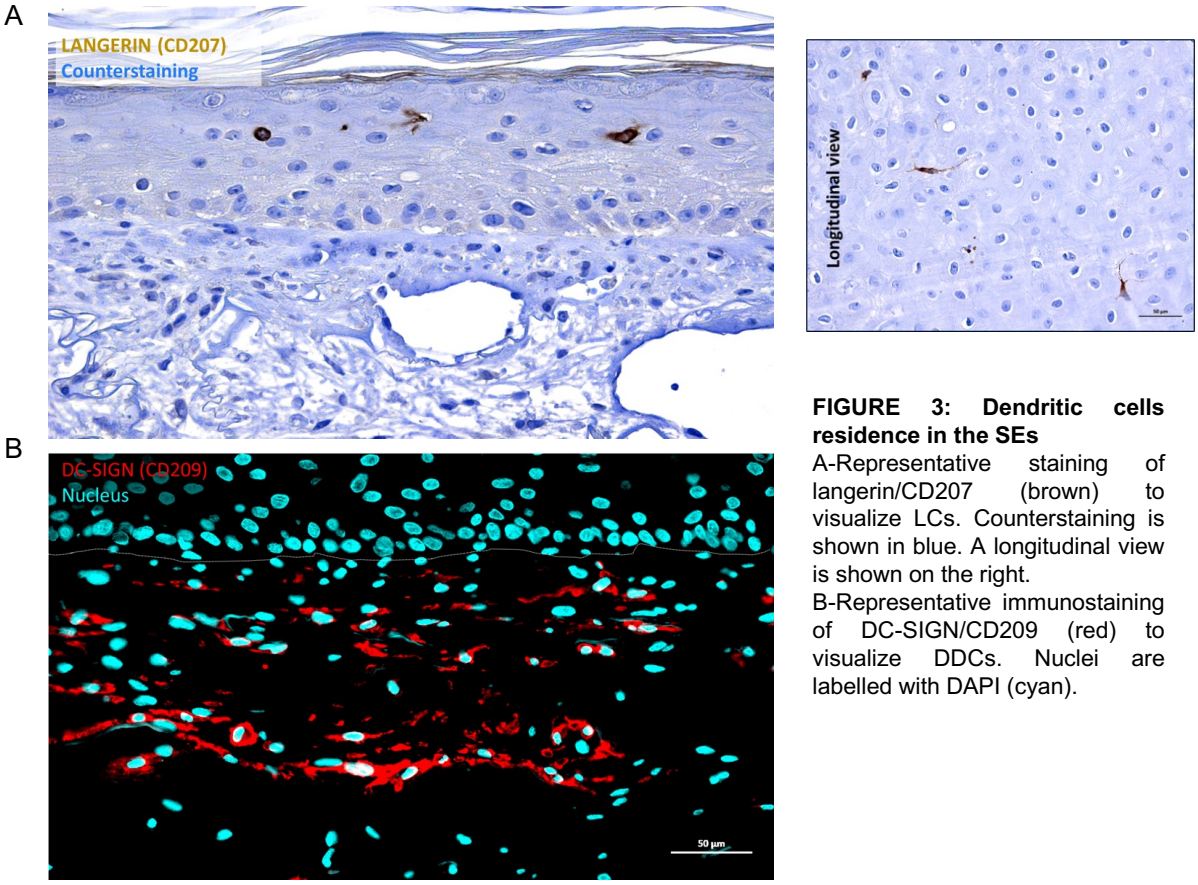


FIGURE 3: Dendritic cells residence in the SEs
A-Representative staining of langerin/CD207 (brown) to visualize LCs. Counterstaining is shown in blue. A longitudinal view is shown on the right.
B-Representative immunostaining of DC-SIGN/CD209 (red) to visualize DDCs. Nuclei are labelled with DAPI (cyan).

LCs and DDCs in SEs are functionally relevant and can be activated by UV stress.

Dendritic cells are sentinels that act as a first line of defense to protect the skin against stressors. They reside in skin compartments, but upon activation they acquire a migratory phenotype to reach the lymph nodes and promote the innate immune response. As our model successfully hosted immune LCs and DDCs, we next assessed whether they were functionally relevant. A combination of UVA and UVB was applied to the SEs at the end of the reconstruction, and the consequences on immune cells assessed after 24 hours. First, we observed that langerin positive cells adopted a migratory morphology with a circular shape and were exclusively detected in the dermis, inside the lumen of capillary-like structure (Figure 4A). In the dermal compartment, DDCs also migrated as DC-SIGN positive cells were mostly found in the deep dermis after UV irradiation (Figure 4B).

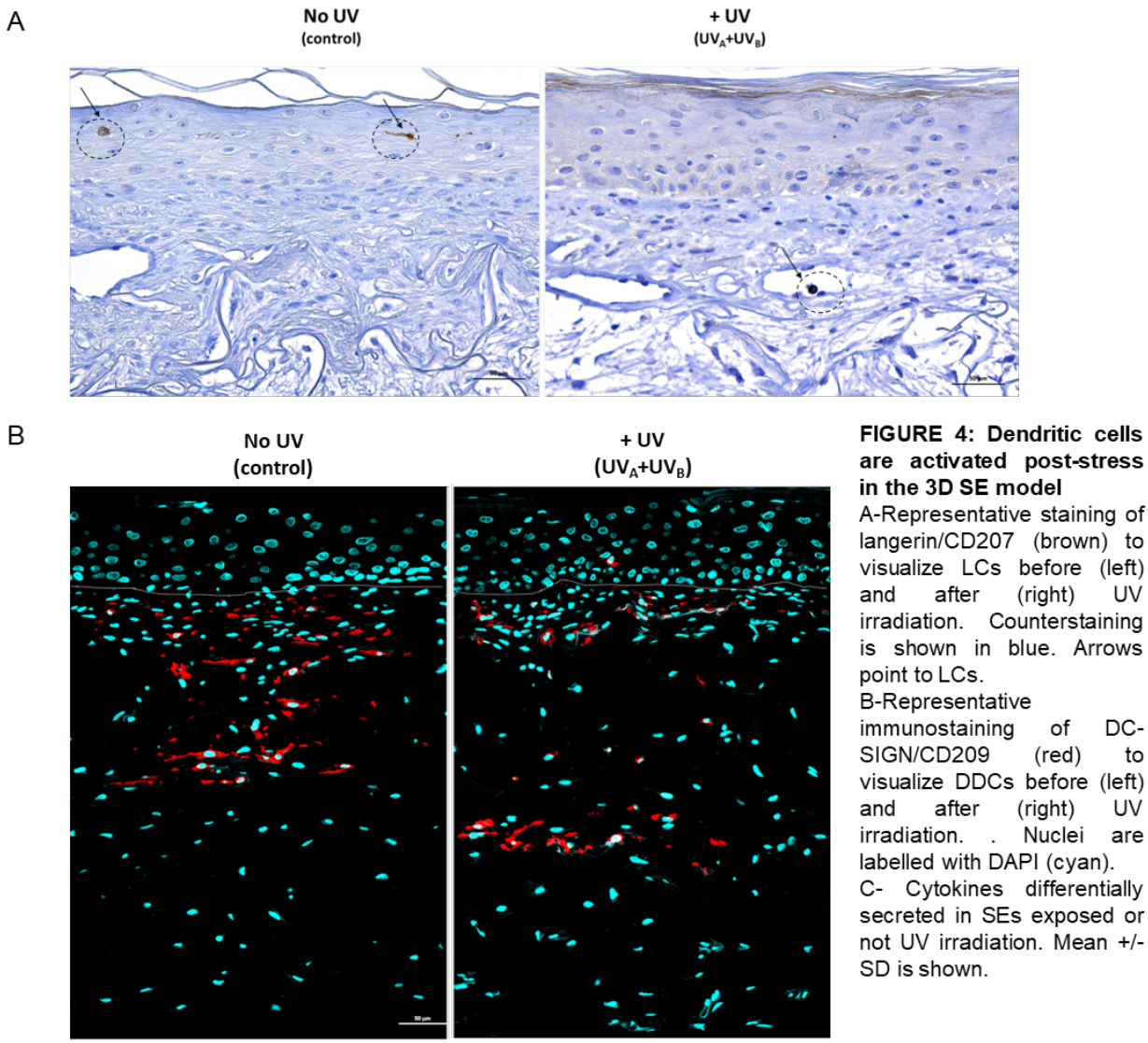


FIGURE 4: Dendritic cells are activated post-stress in the 3D SE model
A-Representative staining of langerin/CD207 (brown) to visualize LCs before (left) and after (right) UV irradiation. Counterstaining is shown in blue. Arrows point to LCs.
B-Representative immunostaining of DC-SIGN/CD209 (red) to visualize DDCs before (left) and after (right) UV irradiation. . Nuclei are labelled with DAPI (cyan).
C- Cytokines differentially secreted in SEs exposed or not UV irradiation. Mean +/- SD is shown.

These results demonstrate that both LCs and DDCs present in the SEs were responding to UV stimulation and that the skin environment allowed for their proper migration, suggesting they could play their role of sentinels in the tissue. We also observed a differential release of

cytokine/chemokine in SEs exposed to UV. An UV-dependent increase of granulocyte colony-stimulating factor (G-CSF), Interleukin 6 (IL-6) and Tumor necrosis factor beta (TNF-beta) was detected, most likely induced by the activation of immune cells. In contrast, two chemokines, fractalkine and interferon gamma-induced protein 10 (IP-10) were found decreased upon UV exposure.

Chronological aging affects LCs and DDCs functionality in SEs

Next, we aimed to determine whether chronological aging could impact the functionality of LCs and DDCs in our SE models. The same tissue engineering technology was applied to construct immunocompetent and microvascularized Human Full-Thickness skins, either using cells from infant (> 5 years old) as before or using cells from an aged donor (57 years old). As expected, the tissue morphology strongly differed between these young and aged models. Aged SEs displayed a much thinner epidermis, some defects in the organization of the epidermal basal layer, and a less dense dermis (Figure 5).

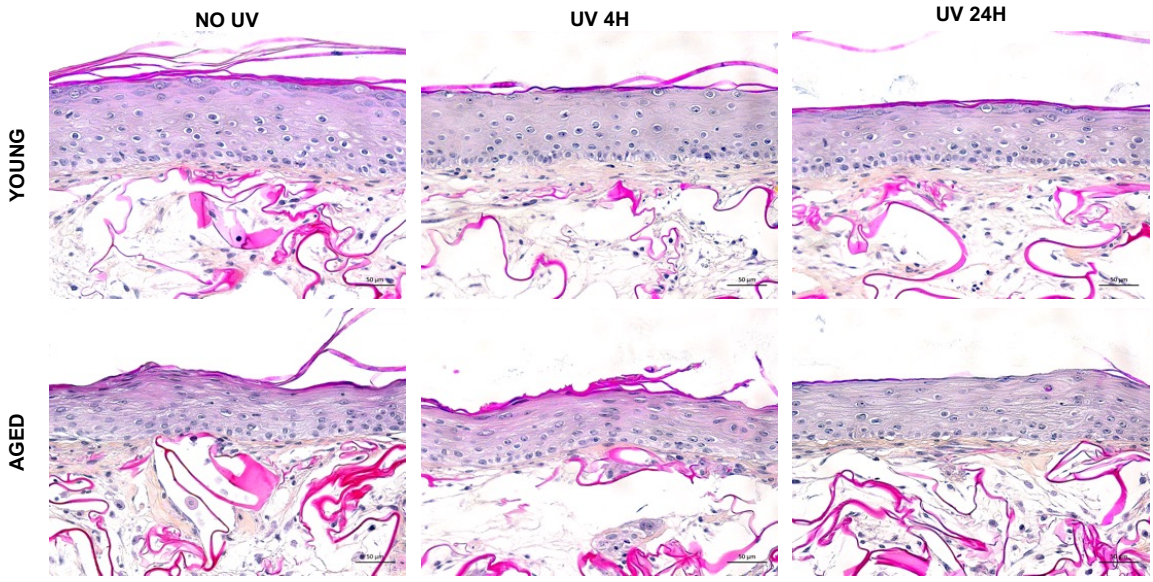
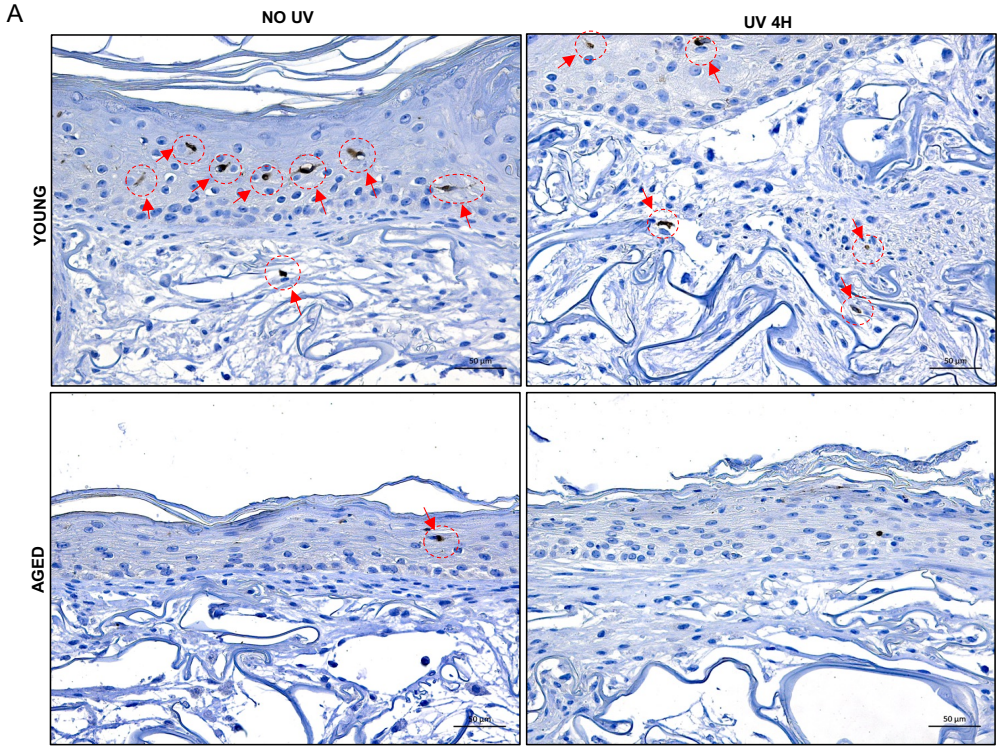


FIGURE 5: Aging impacts immune cells residency and activation following UV exposure
Representative morphology of young SEs (top) and aged SEs (bottom) in control conditions, or 4h or 24h after exposition to UVA+UVB as indicated.

Remarkably, while we confirmed the presence of LCs in the epidermis of young SEs, very few langerin/CD207 positive cells could be spotted in aged SEs (Figure 6). Exposure of both models to a combination of UVA and UVB impacted their morphology, with epidermal thinning, and cohesion defects at the dermal-epidermal junction (DEJ) particularly noticeable in the UV-exposed aged model. While we confirmed the activation of LCs in young models exposed to UV, with langerin-positive cells visualized near the vessels in the dermis, no langerin-positive cells could be detected at the dermal level in the aged model (Figure 6A). These histological observations were confirmed by image processing and analysis showing a drastic decrease of langerin positive cells at the epidermal level in the young 3D SE model when exposed to UV (Figure 6B). Interestingly, our data demonstrated that LC positive cells population was not modulated by UV exposure in the 3D aged suggesting that aging microenvironment was not favorable to the activation of LC positive cells and their migration from the epidermis to the dermis.



B

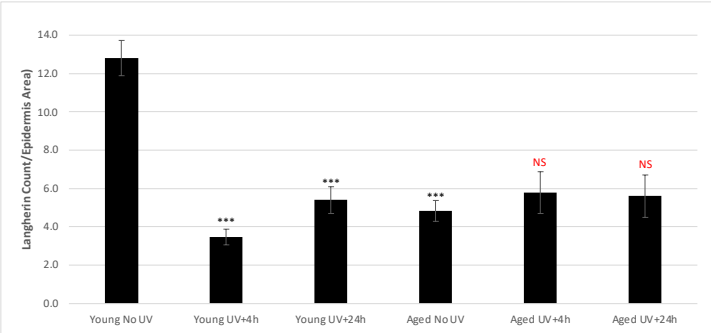
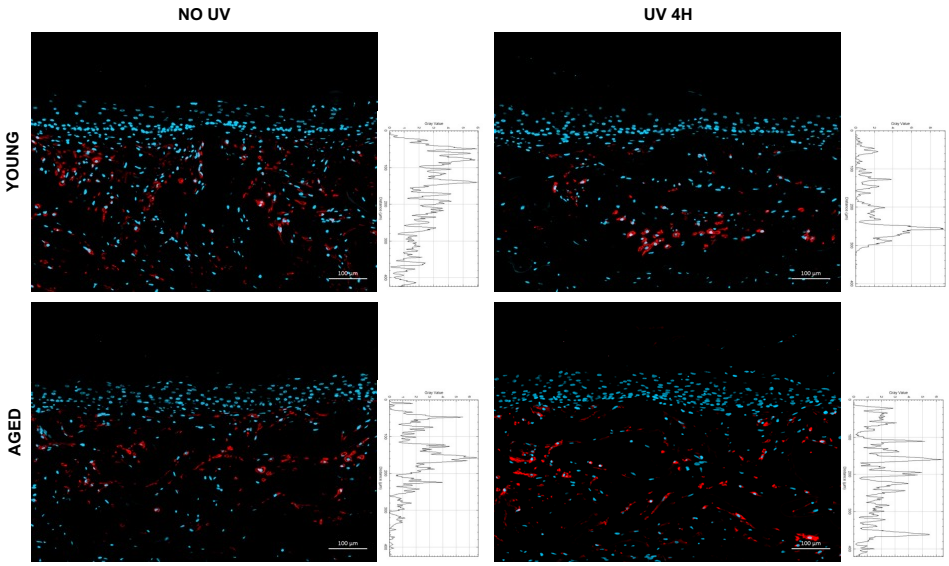


FIGURE 6: Aging impacts langerin positive cells residency and activation following UV exposure
A- Representative staining of langerin/CD207 (brown) to visualize LCs before (left) and after (right) UV irradiation in young SEs (top) and aged SEs (bottom). Counterstaining is shown in blue. Arrows point to LCs.
B. Quantitative analysis by image processing of langerin/CD207 immunostaining in the epidermis. Statistical analysis: young no UV *versus* other conditions in black color; aged No UV versus other conditions in red color.

At the dermal level, a significant decrease of DC-SIGN positive cells was also observed in the dermal compartment of aged SEs compared to the young SEs (Figure 6). Interestingly, in the UV exposed-young SEs, DC-SIGN positive cells were mostly found in the deepest area of the dermal equivalent suggesting their migration from the upper to the deep dermis. This activation was not observed in the UV exposed-aged 3D SEs, in which DC-SIGN positive cells were still homogenously distributed within the dermal area as observed in the aged unexposed 3D SEs.

A



B

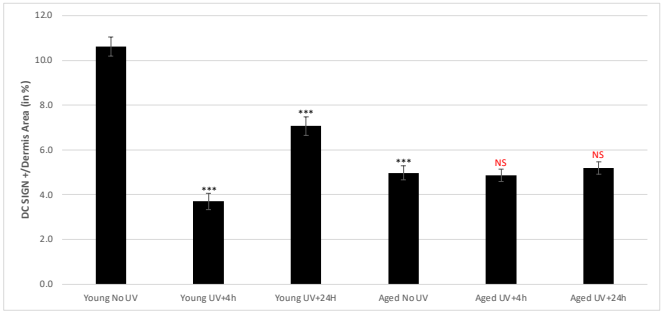


FIGURE 6: Aging impacts DC-SIGN positive cells residency and activation following UV exposure
A- Representative immunostaining of DC-SIGN/CD209 (red) to visualize DDC before (left) and after (right) UV irradiation in young SEs (top) and aged SEs (bottom). Nuclei are labelled with DAPI (cyan). Immune cells distribution within the dermis (from the dermal-epidermal junction to the deep dermis) was quantified by image analysis and processing for each experimental condition.
B. Quantitative analysis by image processing of DC-SIGN/CD209 immunostaining in the dermis. Statistical analysis: young no UV *versus* other conditions in black color; aged No UV versus other conditions in red color.

Altogether these results suggest that an aging skin does not provide the proper environment for immune cells residence and their activation in stress conditions.

Discussion

The generation of human skin models of aging is critical to gain insight into the molecular mechanisms that are altered and test interventions that could favor long-term skin health. Modelling human aging *in vitro* has been a real scientific challenge for many years [6]. While culturing cells in 2D can be useful to characterize cell-intrinsic factors, it does not recapitulate some important features of aging at the tissue level. 3D-reconstructed SEs allow a more in-depth analysis of aging within the skin tissue, the interactions between different layers, consequences of exposure to environmental stress and rescue by treatment with drugs or active ingredients from natural origin [7]. Here, we went one step further and developed an immunocompetent and microvascularized full-thickness skin equivalent to allow for mechanistic and functional studies comprehending the skin immune response. While young SEs harbored resident immune cells that could be mobilized upon stress-induction to reach the skin microvascularization, aged SEs lost these properties. The altered tissular environment in aged SEs did not support LCs and DDCs residence in its epidermal

and dermal layers. As the final differentiation of LC and DDC precursors depends on the cytokine environment [3], one hypothesis is that CD34+ cells did not reach their full differentiation and remained immature. Alternatively, fully differentiated cells might lack the proper skin environment to gain their long-term residency preserved by adhesion to surrounding cells via the expression of adhesion molecules such as E-cadherins.

When young SEs were exposed to a combined dose of UVA and UVB, we observed a mobilization of immune cells that coincided with a stronger release of a set of secreted cytokines. A decrease of two chemokines, fractalkine and IP-10, was also observed. Fractalkine, which has properties of both chemoattractant and adhesion, can have distinct functions in different tissue compartments. It was shown to be expressed by skin epidermal keratinocytes, upregulated upon TNF-alpha and IFN-gamma treatment, as well as overexpressed in lesional skin of psoriatic patients [8]. IC-10 is also secreted by several cell types in response to IFN-gamma and associated with pathologic skin syndromes [9]. Their respective role in human skin is still unclear and could be further investigated. The behavior of aged SEs in response to UV irradiation gave us strong insight on the importance of the skin environment for its immune response.

Conclusion

The resident skin immune system is a rapid and efficient way to respond to stressors. However, skin aging directly impairs its microenvironment and in turn perturbs their functionality. We developed for the first time an immunocompetent and microvascularized 3D skin model to study the impact of both intrinsic and extrinsic aging on skin immune response. We found that skin integrity was a prerequisite for immune cell residence in its layers. When skin was exposed to extrinsic aging factors such as UV irradiation, this altered morphology also affected immune cells behaviour. Altogether, our results strongly suggest the view of aging as a key driver of skin immune response in a complex 3D skin model. Our new immunocompetent and endothelialized skin equivalent may be a relevant tool to further decipher the impact of aging microenvironment on immune cells and identify new cosmetic active ingredients on more physiological basis.

Acknowledgments

We gratefully acknowledge Aksel Bergery and Marie-Océane Chaffois for their technical support.

Conflict of Interest Statement

The authors state no conflict of interest.

References

[1] M. T. Ochoa, A. Loncaric, S. R. Krutzik, T. C. Becker, and R. L. Modlin, "'Dermal Dendritic Cells' Comprise Two Distinct Populations: CD1+ Dendritic Cells and CD209+ Macrophages," *J. Investig. Dermatol.*, vol. 128, no. 9, pp. 2225–2231, 2008, doi: 10.1038/jid.2008.56.

[2] B. Yan *et al.*, "The role of Langerhans cells in epidermal homeostasis and pathogenesis of psoriasis," *J. Cell. Mol. Med.*, vol. 24, no. 20, pp. 11646–11655, 2020, doi: 10.1111/jcmm.15834.

[3] M. J. Toebak, S. Gibbs, D. P. Bruynzeel, R. J. Scheper, and T. Rustemeyer, "Dendritic cells: biology of the skin," *Contact Dermat.*, vol. 60, no. 1, pp. 2–20, 2009, doi: 10.1111/j.1600-0536.2008.01443.x.

[4] A. Achachi *et al.*, "UV Radiation Induces the Epidermal Recruitment of Dendritic Cells that Compensate for the Depletion of Langerhans Cells in Human Skin," *J. Investig. Dermatol.*, vol. 135, no. 8, pp. 2058–2067, 2015, doi: 10.1038/jid.2015.118.

[5] G. Ratzinger *et al.*, "Matrix Metalloproteinases 9 and 2 Are Necessary for the Migration of Langerhans Cells and Dermal Dendritic Cells from Human and Murine Skin," *J. Immunol.*, vol. 168, no. 9, pp. 4361–4371, 2002, doi: 10.4049/jimmunol.168.9.4361.

[6] A. Brunet, "Old and new models for the study of human ageing," *Nat. Rev. Mol. Cell Biol.*, vol. 21, no. 9, pp. 491–493, 2020, doi: 10.1038/s41580-020-0266-4.

[7] M. Albouy *et al.*, “Skin-protective biological activities of bio-fermented Aframomum angustifolium extract by a consortium of microorganisms,” *Front. Pharmacol.*, vol. 14, p. 1303198, 2023, doi: 10.3389/fphar.2023.1303198.

[8] M. Sugaya, K. Nakamura, H. Mitsui, T. Takekoshi, H. Saeki, and K. Tamaki, “Human keratinocytes express fractalkine/CX3CL1,” *J. Dermatol. Sci.*, vol. 31, no. 3, pp. 179–187, 2003, doi: 10.1016/s0923-1811(03)00031-8.

[9] J. M. Richmond *et al.*, “Keratinocyte-Derived Chemokines Orchestrate T-Cell Positioning in the Epidermis during Vitiligo and May Serve as Biomarkers of Disease,” *J. Investig. Dermatol.*, vol. 137, no. 2, pp. 350–358, 2017, doi: 10.1016/j.jid.2016.09.016.