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T-SKIN™, A NEW INDUSTRIAL RECONSTRUCTED FULL THICKNESS SKIN MODEL AND ITS COSMETIC APPLICATIONS

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ABSTRACT

In vitro reconstructed skin models are useful tools for safety and efficacy studies. The industrial reconstructed human full thickness skin model T-Skin™ is composed of a living dermal equivalent (human fibroblast-contracted collagen gel) and a well-stratified and fully differentiated epidermis (human keratinocytes). Moreover, immunohistochemistry characterization studies showed that T-Skin™ presents the well-known located epidermal and dermal biomarkers (Cytokeratin 10, Procoll-I, Collagen-IV…) as described in the *in-vivo* normal human skin. Thanks to numerous biological endpoints related to *in vivo* biological response and its functionally 3D skin structure, the full-thickness T-Skin™ model is a promising tool for understanding specific biological effects of UV exposure, ageing and oxidative stress mechanisms, reflecting real situations. Thus, different *in vitro* protocol approaches were developed on T-Skin™, allowing, for example, to identify photoprotection efficacy, anti-ageing or antioxidant properties of cosmetic products. Taken together, these results confirm that the industrial T-Skin™ model represents a relevant and powerful tool in a screening platform to develop new cosmetics and dermatological active ingredients.

INTRODUCTION

Mechanisms of skin chronological or photo - ageing involve biological pathways associated to alterations of the epidermis as well as dermis compartments. These mechanisms are complex to study and the access to biopsies is more and more difficult. The reconstructed 3D skin model gives us the possibility to study parameters linked to *in vivo* ageing and photo-ageing mechanisms. In the cosmetic domain, the epidermal reconstructed models offer the opportunities to mimic some aspects of clinical studies and to predict the efficacy of active ingredients or finished cosmetic products. Integration of living dermis allows to study these types of mechanisms and to develop new cosmetic active ingredients specific to each biological response. Therefore, we developped a new 3D industrial full thickness model named T-Skin™.

This poster presents immunohistochemistry characterizations of intracellular and extracellular well-known biomarkers located in epidermis, dermal-epidermal junction and dermis compartment of T-Skin™ in comparison to normal human skin (NHS). Functional responses were characterized after treatment with anti-ageing reference molecules such as Vitamin C (L-ascorbic acid) and retinol or after long UVA exposure associated to Vitamin C treatment.

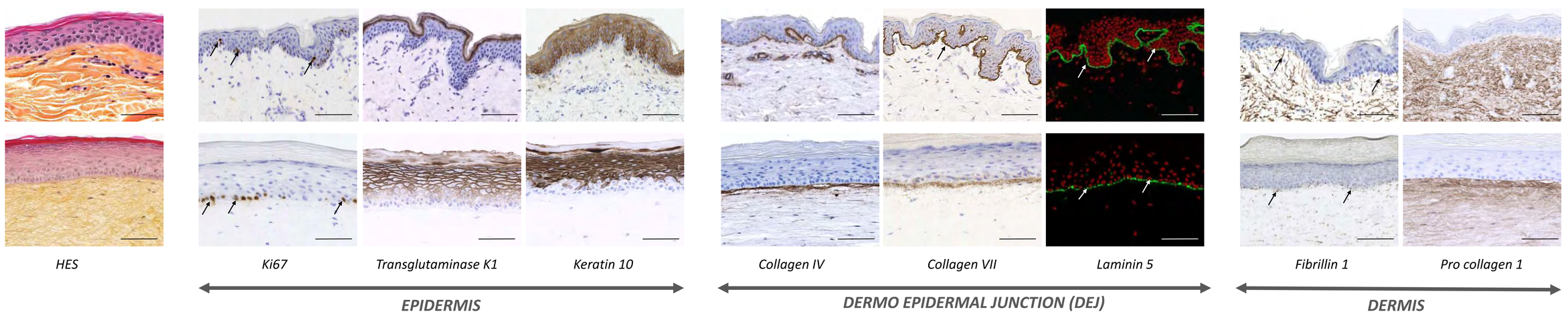
MATERIALS & METHODS

Reconstructed T-Skin culture

NHS were obtained from mammary plastic surgery after patients informed consent. Keratinocytes and fibroblasts were extracted and prepared.
T-Skin™, a human full thickness skin model from EPISKIN SA consists of a living dermal equivalent with a lattice of native collagen type I containing normal human fibroblasts cultured for few days. This dermal equivalent was incubated with fresh appropriate medium at 37° C under 5% CO2. Then, keratinocytes were seeded at the top of the dermal lattice and maintained in the appropriate culture medium at 37° C under 5% CO2 for epidermis reconstruction.
A well-stratified and fully differentiated epidermis is obtained after 3 weeks of incubation (manufacturing under ISO9001 quality system).

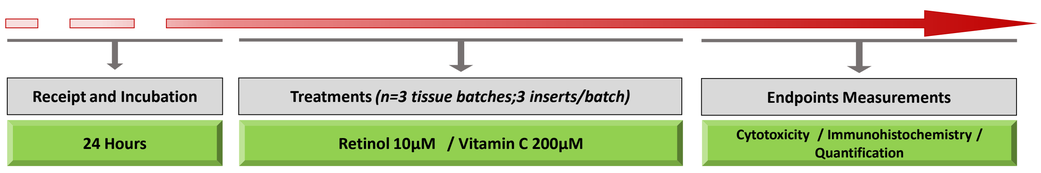
Primary antibodies : [paraffin sections] transglutaminase 1 (clone BT-621, Biomedical Technologies), keratin 10 (clone DE-K10, Agilent), Ki67 (clone MIB-5, Agilent), collagen IV (clone CIV 22, Agilent), fibrillin (clone 26, Millipore), pro-collagen I (clone 2Q576, Abcam); [cryostat sections] laminin-5 (clone D4B5; Millipore, Billerica, MA, USA), keratin 10 (clone RKSE60; Monosan, Uden, Netherlands), collagen IV (clone CIV 22; Agilent, Santa Clara, CA, USA), collagen VII (clone 4D2; Santa Cruz, CA, USA), pro-collagen I (clone M-58; Millipore, Billerica, MA, USA) and secondary antibody conjugated to Alexa488® (Life Technologies, Grand Island, NY, USA). Nuclei were stained with propidium iodide solution (Sigma-Aldrich, St Louis, MO, USA).
Reference molecules: vitamin C (113170-55-1; Sigma-Aldrich) and retinol (68-26-8; Sigma-Aldrich) were solubilized in water or DMSO 10% respectively.

T-SKIN IMMUNOHISTOLOGICAL CHARACTERIZATION

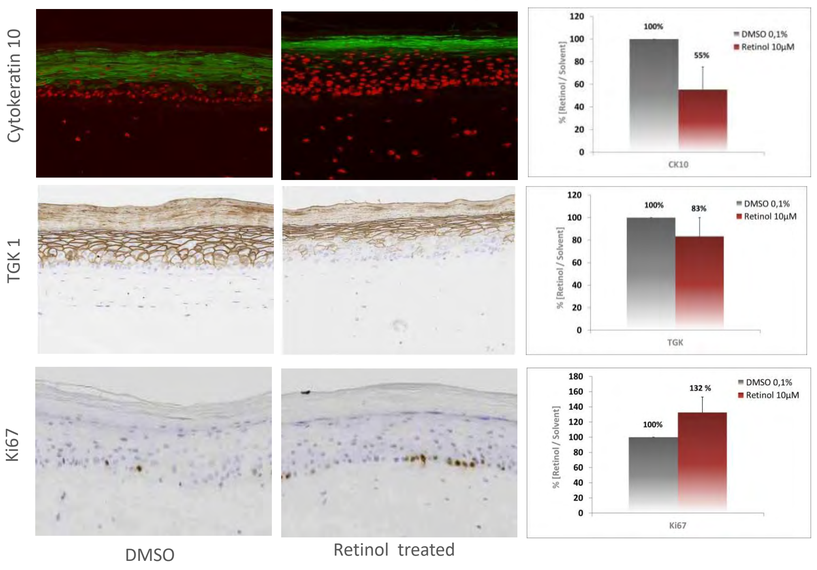


Bars: 100µm, arrows indicate specific labelling

ANTI-AGEING PROPERTIES OF COSMETIC COMPOUNDS

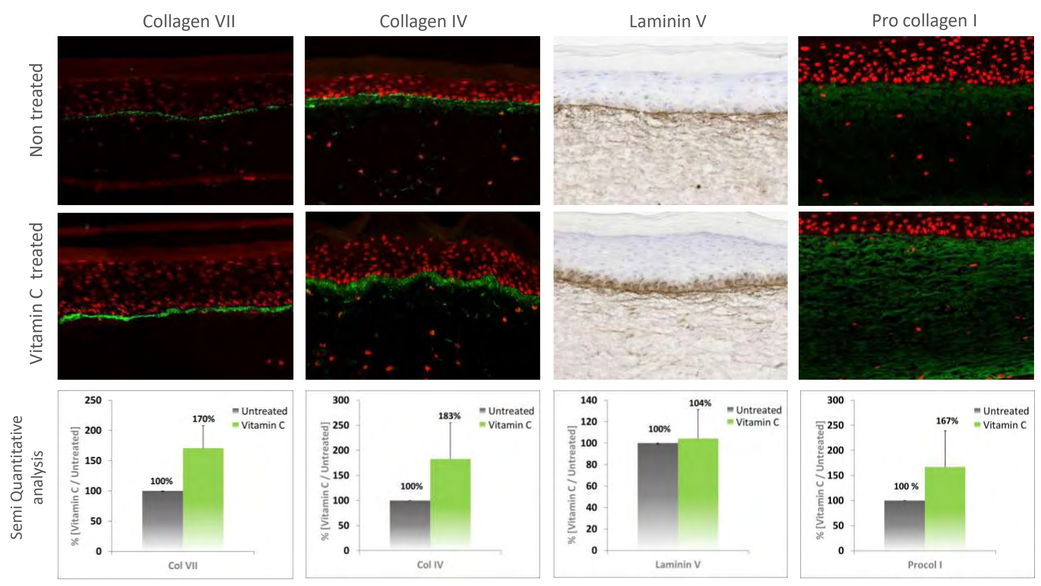


Epidermal response of T-Skin™ after 5 days Retinol treatment



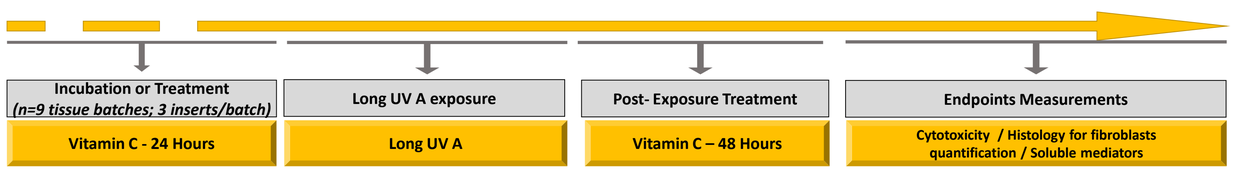
Our results show that after 5 days of contact with retinol at 10µM, we observed a relocalization of Cytokeratin 10 and Transglutaminase 1 expression compared to DMSO condition. Indeed, the immune labelling is detected in the epidermal granulosum layer instead of granulosum and spinosum layers with control condition. The semi-quantitative scoring method (0: no staining to 4: maximal staining; objective X10) confirms the decrease of their expressions with a 45% and 17% respectively for Cytokeratin 10 and Transglutaminase 1. In parallel, we observed an increase of 32% of the Ki67 positive cells in the basal layer of T-Skin™ epidermis. These results confirm the key role of retinol treatment on the cutaneous proliferation and differentiation process.

DEJ and dermal response of T-Skin™ after 5 days Vitamin C treatment

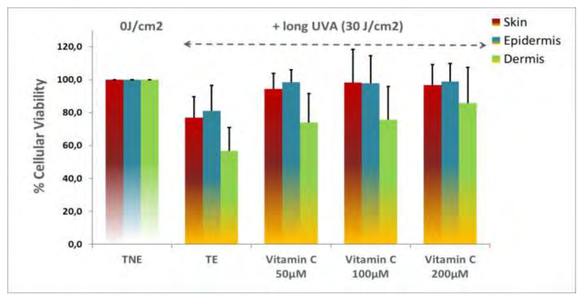


A strong immunolabelling, evaluated by semi-quantitative scoring as previously described (objective X10) is observed after treatment of T-Skin™ tissues during 5 days with 200µM of vitamin C. Collagen IV and VII expression is respectively increased from 70 and 83%. The quantification of Laminin-V is not increased with a similar level but the microphotographies reveal a diffusion of the labelling on each side of the DEJ. Expression of the procollagen I after vitamin C treatment is increased of 67% compared to untreated condition. The microphotography reveals the localization at the dermal superficial part which could be assimilate to the papillary *in vivo* dermis. Altogether, these results confirm the well-known activity of vitamin C on the extracellular matrix synthesis in the T-Skin™ full thickness model.

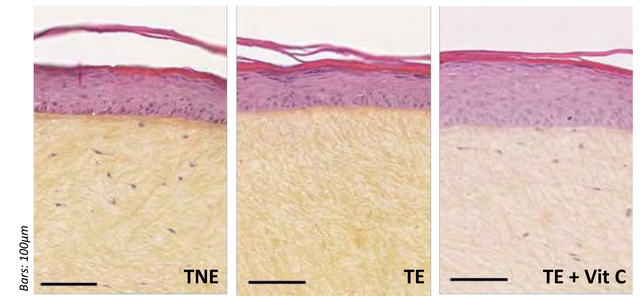
PHOTO PROTECTIVE PROPERTIES OF COSMETIC COMPOUND



Vitamin C Phototoxicity evaluated by MTT analysis

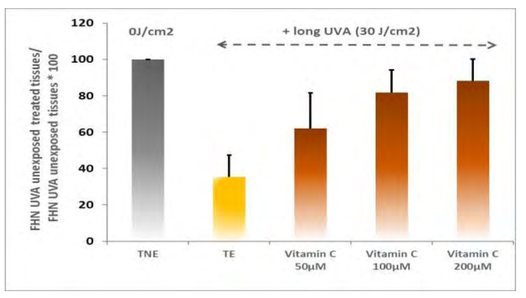


Dermal Damages evaluation

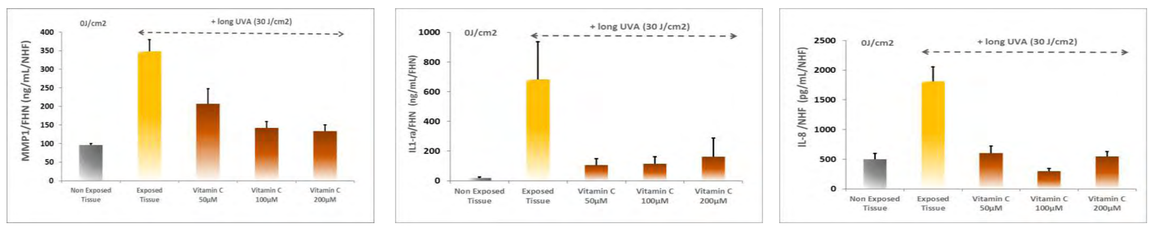


HES histology studies show the presence and appropriate repartition of fibroblasts in the dermis of T-Skin™ tissues in non-exposed condition (TNE; 0 J/cm2). After the long UVA dose of 30J/cm² exposure (TE), a strong disappearance of dermal fibroblasts of T-Skin™ tissues is observed without significant epidermal cellular alteration. The dermal viability strongly decreases at 30 J.cm2 dose (more than 45%). A slight decrease of the viability (<20%) is observed for the epidermal part.

As expected, Vitamin C protects the skin against long UVA damages effect on dermis (viability decrease and fibroblast disappearance) as shown on the graph beside by following a dose effect. The maximal effect is visible at 200µM. Histology HES approach confirms the strong photo protective effect of Vitamin C on dermis integrity against UVA exposure damages (no fibroblast disappearance for vitamin C treated and exposed tissues).



Vitamin C protective effect on pro-inflammatory response



MMP1 IL1-ra IL8

The skin response to UV exposure, specifically long UVA wavelength, is characterized by strong releases of metalloproteinase MMP-1 and different cytokines such as IL-1α, IL-8, IL-6, GM-CSF or IL-1ra. In the present study, we focused on MMP-1 and the 2 major cytokines, IL-1ra and IL-8.

Exposure of T-Skin™ tissues at 30J/cm² of long UVA, induces a strong increase of MMP1 and cytokines IL-1ra and IL-8 release compared to non-exposed tissues. In contrast, Vitamin C concentrations limit MMP-1 and Cytokines amounts in the culture medium after exposure. Similar results were obtained for the cytokines, IL-1α, IL-6 & GM-CSF (data not shown).

CONCLUSION

Our industrial reconstructed human full thickness skin model T-Skin™ is a well-characterized model with good reproducibility. T-Skin™ shows a high correlation with *in vivo* normal human skin both in terms of organization and in response to different stimuli.
This model is a promising tool for understanding specific biological effects of UV exposure, ageing and oxidative stress mechanisms, reflecting real situations. It represents a relevant and powerful tool in a screening platform to develop new cosmetics and dermatological active ingredients.
This model could also help us to thwart the decrease of access to human biopsies.