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Skin CHALLENGES

LONG-TERM SKIN TISSUE MAINTENANCE FOR THE BENEFIT OF PRODUCT EFFICIENCY

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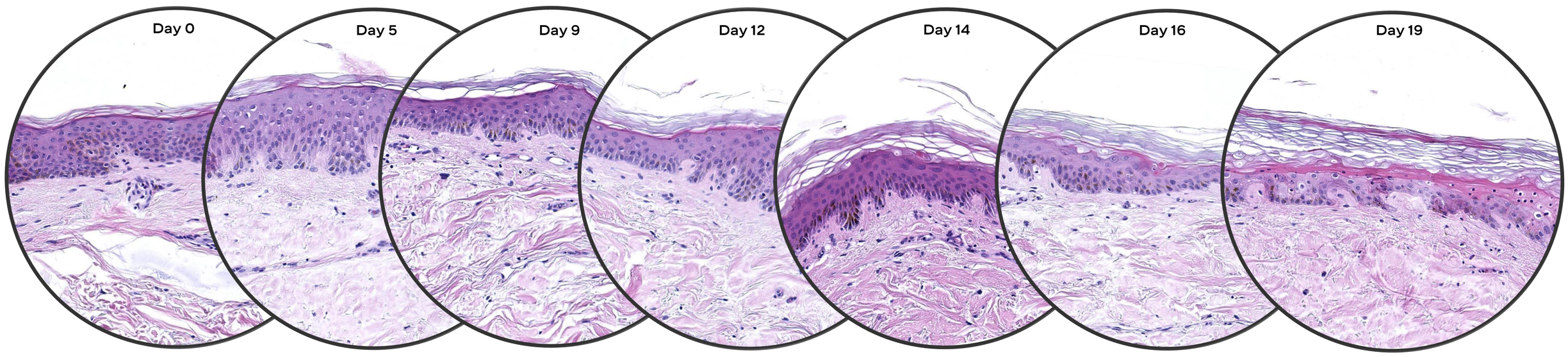
INTRODUCTION

Skin acts as a protective barrier against external aggressions that our bodies are submitted to on a daily basis, although it can also be challenged by endogenous stresses. 3D reconstructed skin models are often used in the cosmetic industry as an alternative to in vivo animal models, but although very reproducible, they do not cover the entire complexity of the morphological and physiological characteristics of human skin. Ex vivo can be a more complex and complementary research model helping to understand skin responsiveness to internal & external factors (UV...) and to evaluate the effect of active ingredients and cosmetic products on biological mechanisms. Here we present an innovative ex vivo method consisting in a dynamic maintenance of human skin samples in culture for up to 19 days, that allows us to study skin response to environmental stresses or cosmetic products over a long period of time. This long-term culture makes it possible to adapt the culture time to the biological theme studied and allows to ensure perfect tissue viability on the window of use of our model for common studies. For example, up to 3 days to evaluate the exfoliating nature of a product, up to 10 days to evaluate the anti-aging effectiveness...

So, the development of this model up to 19 days is a proof of quality and allows us to be confident about the results delivered on the window of use of our explants for common studies. Through this presentation, we highlight the use of our ex vivo model to prove the exfoliating effect of a topically applied product, The Lotion. Finally, the quantification of the observed effect was done using specific "soft ware" tools where AI & deeplearning were implied.

1/ DYNAMIC MAINTENANCE OF HUMAN SKIN EXPLANTS FOR UP TO 19 DAYS

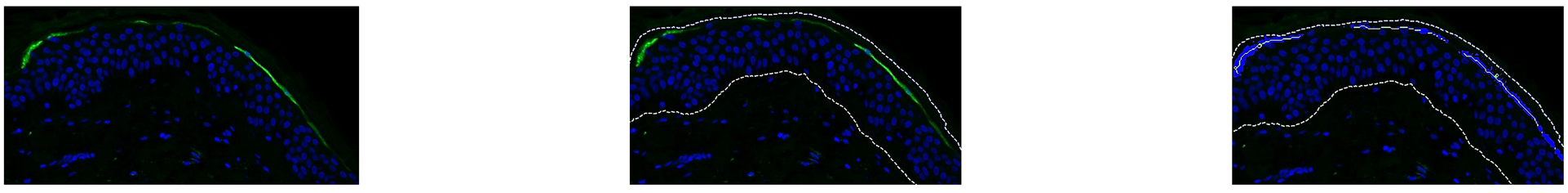
METHOD: An abdominoplasty from a 61-year-old woman was treated to remove the hypoderm. Punches of 12mm were taken out of the skin sample and placed on porous cell culture inserts, which were disposed over the wells culture plate containing an internally-developed culture medium for up to 19 days, at 37°C +/-2°C in a humid atmosphere at 5% CO2 . The medium was removed and changed every two days. At every day of assessment, skin samples were put into histological cassettes and immersed overnight into a 10% Formalin solution at 4°C. The next morning, skin tissues were dehydrated through several baths of increased concentrations of ethanol and xylene, followed by liquid paraffin baths using the Histocore PEARL (Leica). This step was followed by paraffin embedding of the samples. Paraffin-embedded samples were then cut with a semi-automatic microtome (Leica) set at 4µm. Sections were put into a 42°C waterbath until they unfold and were then placed on a Superfrost plus histological glass slides (Epredia), then dried in an oven at 37°C overnight. Sections were then deparaffinized and stained with Hematoxylin-Eosin staining using an automatic Stainer (Leica), mounted in Eukitt mounting media and covered with coverslips. Slides were scanned in brightfield at 20X with an Aperio Versa (Leica) scanner.



RESULTS: Until day 16, the quality of the epidermis is good; there are no vacuoles, the basal layer is well organized, and the differentiation of epidermis is of good quality. At day 19, vacuoles start to appear and there are signs of hyperproliferation in the granular layer. This model can therefore be used as a robust way to explore skin physiology, stress response and cosmetic products efficacy, at least until day 16.

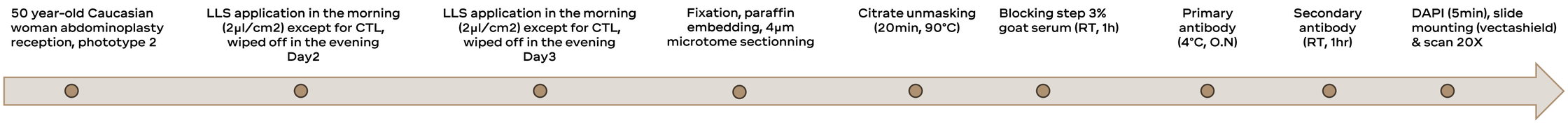
2/ USING DEEPLEARNING ALGORITHMS TO ENHANCE THE VALUE OF RESULTS

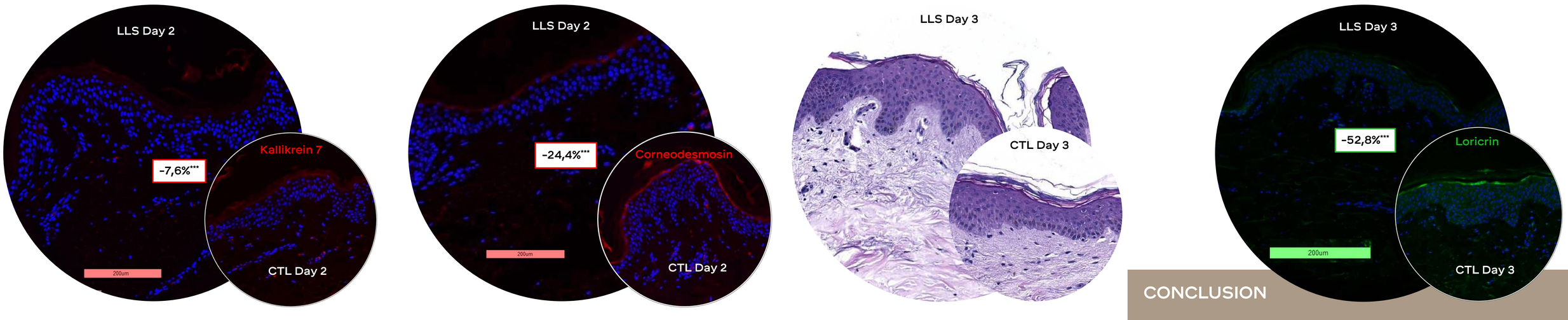
We used the artificial intelligence software Visiopharm® to analyze our images. For each immunostaining, a specific application was developed in collaboration with Visiopharm® to detect the target of interest. After training the application on multiple images, we obtained a two-step way to analyze biomarkers located in the epidermis: the first step consists of segmenting the tissue based on the recognition of nuclei stained with DAPI and outlining the epidermis as a region of interest; the second step is to detect the biomarker within the previously outlined epidermis, in the channel used for staining, based on a pixel threshold method. The output variables can be chosen to fit the need, for barrier function proteins we chose to quantify the mean intensity and the length of the staining.



3/PROOF OF EFFICACY OF THE EXFOLIATING EFFECT OF 'The LOTION'

METHOD: Exfoliation involves removing dead cells from the surface of the skin. To evaluate the effectiveness of an exfoliating product it is necessary to look at markers of tissue differentiation without effect on tissue viability. The first step consists of verifying that the product does not alter the viability of the skin by HE staining and then performing fluorescent immunostaining directed towards terminal differentiation. If the product (LLS) is well exfoliating, then the reduction in these markers Corneodesmosin, Loricrin and Kallikrein 7, compared to the untreated control condition (CTL) will be observed. The following timeline outlines the different steps required to obtain an immunostaining:





RESULTS: The HE staining allows us to confirm that the Lotion does not impact the viability of tissue but with different biomarkers we can observe a significant decrease of corneodesmosin by 24,4%***, kallikrein 7 by 7,6%*** after 2 days of application, and loricrin for which the decrease by 52,8%*** takes longer to take place, after 3 days of application, compared to untreated explants. These results highlight the exfoliating effect of the product. All statistical analysis were performed on data obtained on experimental triplicate, a Mann Whitney Wilcoxon was performed to compare treated and control conditions for each immunostaining.

CONCLUSION

Our innovative model gives us a robust way to assess the efficacy of our products in the more or less long term. Artificial intelligence allows us to quantify the efficacy of our products with the aim of moving towards even greater performance.