PATTERNED HUMAN EPIDERMAL VITRO MODEL USING BIOPRINTING TO MIMIC HETEROGENEITY OF HUMAN SKIN

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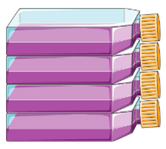
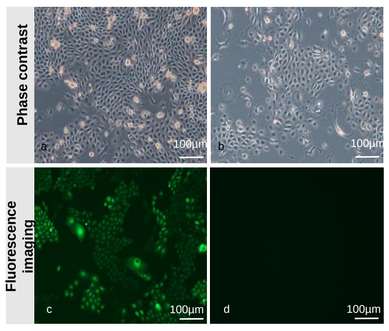
1 INTRODUCTION

Human skin is highly heterogeneous across individuals, anatomical sites and during skin pathology. Human skin models have been used for decades in dermato-cosmetic evaluation tests but they represent only one state of the skin and not this heterogeneity on a single model resulting in limited predictivity when it comes studying the interactions between different types of skin tissue. This study aims to use bioprinting technology to deposit in a controlled manner two populations of human keratinocytes mimicking some heterogeneities of Atopic Dermatitis (AD) combining healthy skin zones and lesional zones.

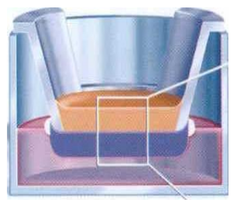
2 MATERIALS AND METHODS



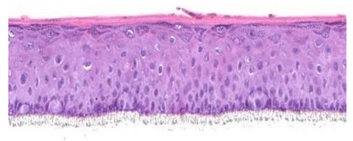
Foreskin primary normal human keratinocytes (NHK)



Transduction of NHKs with shLUCIFERASE-GFP (control) or shFLG

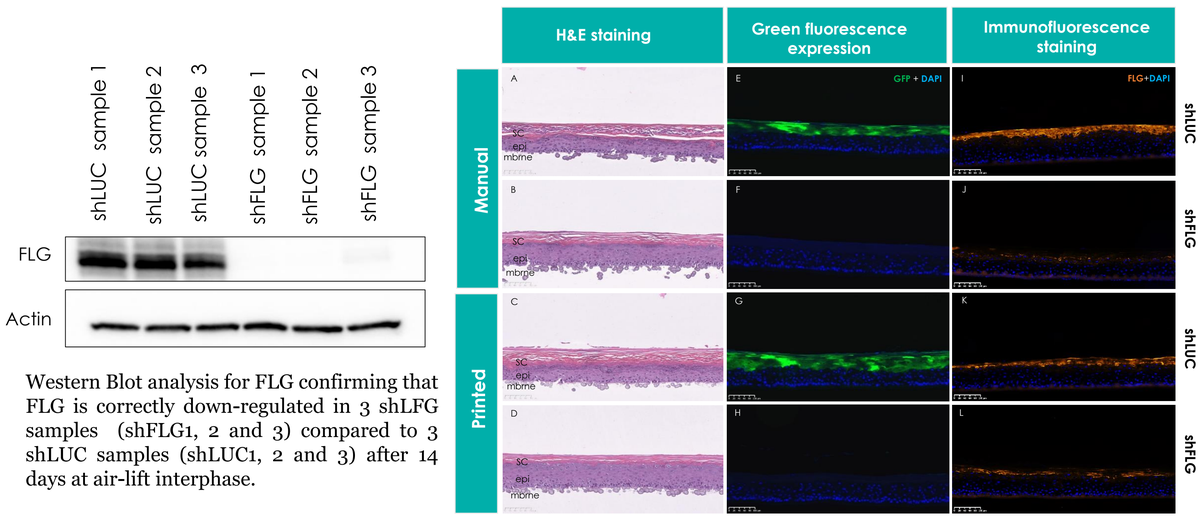


Tissues were cultured **at air-liquid interface 14 days**



Tissues were **harvested** and underwent **histological, fluorescence** and **immunostaining analysis**

3 RESULTS & DISCUSSION



Western Blot analysis for FLG confirming that FLG is correctly down-regulated in 3 shLFG samples (shFLG1, 2 and 3) compared to 3 shLUC samples (shLUC1, 2 and 3) after 14 days at air-lift interphase.

Figure 1. Tissue engineered RHE reconstructs were obtained using shLUC-transduced (A, C, E, G, I *and* K) and shFLG transduced (B, D, F, H, J *and* L) NHKs. Morphological analysis was done by H&E staining (A, B, C and D), Green fluorescence expression (E, F, G and H) and immunofluorescence staining on Filaggrin (FLG) (I, J K and L) after 14 days of culture at air-liquid interface. In order to assess any potential machine-dependent effect on the RHE reconstructs, samples were seeded by conventionally pipetting the cellular suspension onto the culture support (A, B, C, G, H *and* I) and the others were directly printed onto the culture membrane (D, E, F, J, K *and* L). Images show that all histology are corrects with presence of all differentiation layers in the shLUC samples and hypogranulosis in the shFLG samples as expected. Green fluorescence is present only in the shLUC samples and FLG is correctly down-regulated in shFLG samples.
Sc : stratum corneum ; epi : epidermis ; mbrne : polycarbonate membrane support

Bioprinted shLUC and shFLG epidermal models keep their phenotypes

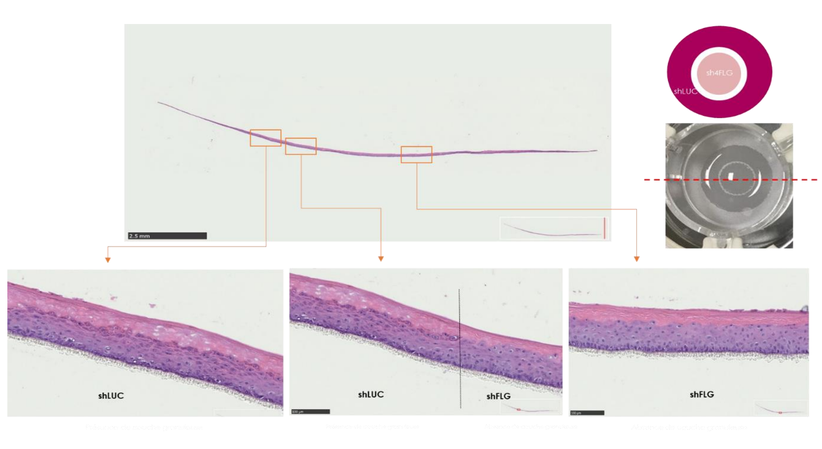


Figure 2. *Histological analysis of shLUC and shFLG parts of pattern bioprinted reconstructed skin. The shLUC parts of the patterned model are histologically normal as anticipated, which abruptly ceases at the border with the shFLG printed center. The shFLG printed center of the model demonstrates a lack of keratohyalin granules demonstrating the model to be accurately printed and stable with no migration of cell populations after 14 days in culture. Images representative of 8 individual samples.*

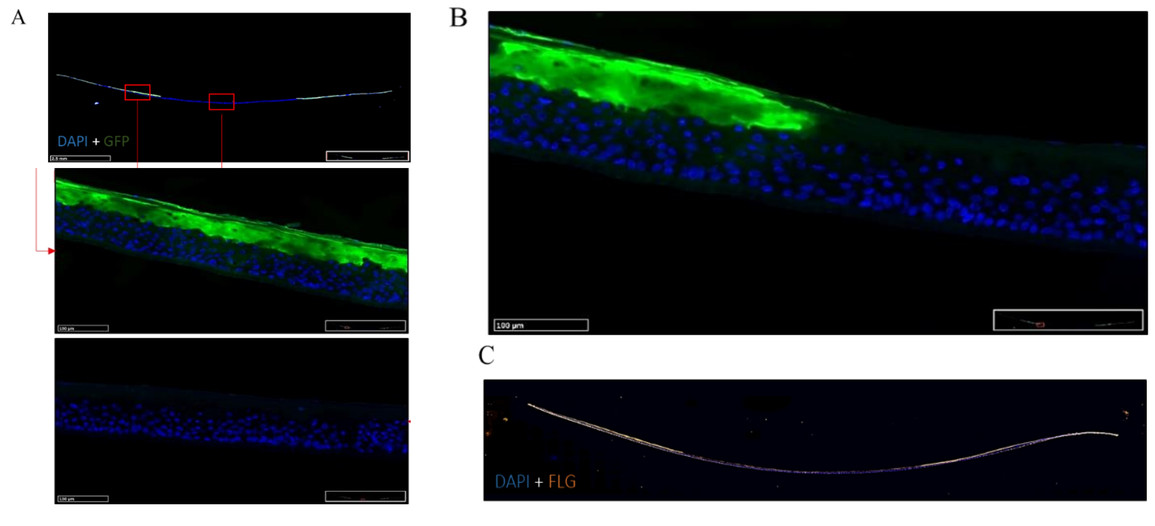


Figure 3. *GFP expression and immunofluorescence of fillagrin (FLG) in shLUC and shFLG parts of pattern bioprinted reconstructed skin. (A) The suLUC parts of the patterned model show high levels of GFP staining as anticipated, which abruptly ceases at the border with the shFLG printed center (**B**) while the shFLG printed center of the model has no GFP signal. (B) FLG expression is strongly express in the shLUC parts and downregulated in the shFLG center part of the reconstructed skin. This demonstrates that the model can be accurately printed and stable with no migration of cell populations after 14 days in culture. Images representative of 8 individual samples.*

Bioprinted RHE constructs were achieved using shLUC and shFLG on a same sample in order to pattern the concentric-circles design.
Populations of keratinocytes formed a homogeneous tissue following predefined designs

4 CONCLUSIONS

This is the first report of human patterned epidermal model using a predefined bioprinted designs and demonstrates the relevance of bioprinting to faithfully reproduce human skin microanatomy. This robust proof on concept on an existing model of AD, could be applied to other dermatological skin model as precancerous or eczema lesions but also to models of interest for cosmetic as transition between lips and face, scalp and face or pigmented spots. Pattern models will help us to understand communications between different skin states but also to test a raw material or drug of interest on different skin conditions simultaneously.

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References:

1. Pendaries, V. et al. Knockdown of filaggrin in a three-dimensional reconstructed human epidermis impairs keratinocyte differentiation. J Invest Dermatol 134, 2938-2946, doi:10.1038/jid.2014.259 (2014).