Skin regeneration on FT-skin

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INTRODUCTION

Skin regeneration is a physiological and complex process involving different cell types to restore tissue continuity after wound: activated keratinocytes migrate from the wound edges to generate a provisional wound bed matrix where they can proliferate, differentiate, and stratify into a new epidermis. The complete skin repair process involves fibroblasts activation and ECM remodeling.
The aim of this project was to develop an *in vitro* experimental model on commercially available 3D reconstructed Full Thickness skin (FT-skin) able to recapitulate skin regeneration dynamics over time.
The wound healing model described in this work has been further developed by VitroScreen within the project EUROSTARS E! 113238 aimed at developing a new model that takes into account *S. aureus* infecting diabetic wounds.

MATERIALS & METHODS

Phenion® Full Thickness Skin Model produced by Henkel (Düsseldorf, Germany, diameter of 1.4cm and surface area of 1.5cm^2) are cultured from keratinocytes and dermal fibroblasts derived from biopsy material from a single healthy donor to form a multi-layered skin equivalent that resembles human skin. Each FT-skin tissue was injured in the centre with a sterile 1 mm biopsy punch (Fig. 1) to create a physical discontinuity in both epidermis and dermis layers and subsequently cultured for up to 20 days.

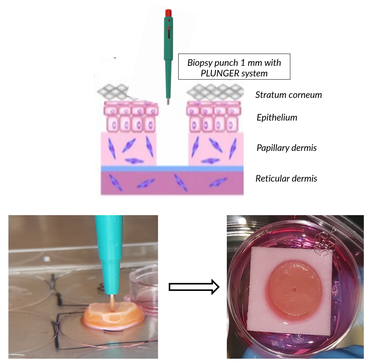

Fig. 1 Schematic representation of the injury procedure in a FT-Skin model.

At defined timepoints (0, 1, 3, 8, 20 days), FT-skin tissues were fixed in 10% buffered formalin (Sigma Aldrich, HT501128) and embedded into paraffin blocks to obtain sections of 5 µm. Slides were stained with H&E and immunolabeled (IHC/IF) with biomarkers reported in Tab I:

Protein target	Primary Ab Code	Supplier
Cytokeratin 14 (CK14)	ab7800	Abcam
Cytokeratin 10 (CK10)	HPA012014	Sigma Aldrich
Fibronectin (FN)	ab2413	Abcam

Tab 1. Primary antibodies list

All secondary antibodies were diluted 1:400 in PBS 1x. DAPI was used for nuclei staining. Images acquisition was performed by LEICA DMi8 THUNDER imager 3D System (Leica) composed by camera sCMOS K5 and LASX software 3.7.5.

RESULTS

Tissue repair dynamics were investigated by Hematoxylin and Eosin (H&E) (Fig. 1) from Day 0 to Day 20.

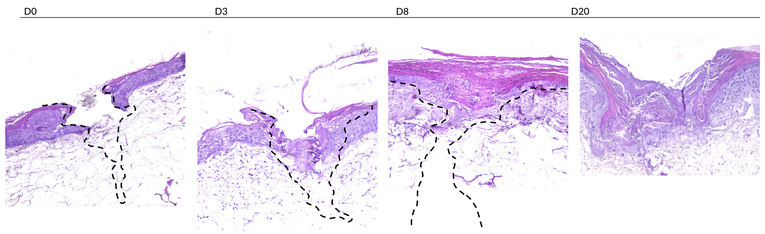

Fig 1. H&E staining shows morphological variation and progressive closure of wounds from day 0 (d0) up to day 20 (d20) from injury. Magnification 10X.

The epidermis seamlessly attached to the dermis compartment except in the wound area on day 0 (D0), providing an *in vivo*–like matrix for re-epithelialization surrounded by clearly defined wound margins. Tissue continuity is destroyed in both epithelial and dermal compartment. The extending epidermal tongue is visible as 1-2 cell layers in the wound bed on day 3 (D3): the presence of cells randomly distributed in the wound bed suggest formation of new tissue. The keratinocytes covered 100% of the wound area on day 8 (D8) by organizing the epithelium into approximately three cell layers near the wound edges and one cell layer in the centre of the wound bed. The dotted line indicates the boundary between epithelium and dermis: keratinocytes are still visible in the wound bed. On day 20 it is possible to appreciate the dermal remodelling.

Keratinocytes differentiation during re-epithelization was investigated by IF for localization and expression of CK10 and CK14 in the newly formed epidermis at Day 1, 3 and 8 (Fig. 2). CK10 was used as differentiation biomarker while CK14 as marker for undifferentiated basal keratinocytes.

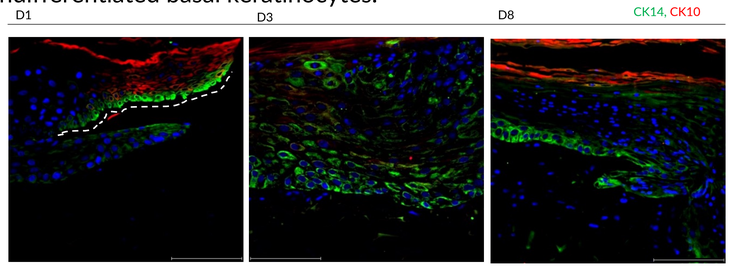

Fig 2. IF of CK14 and CK10 on the wounded bed on Day 1, Day 3 and Day 8. Nuclei are stained with DAPI (blue). Magnification 20X. Scale bar: 100 µm.

The early expression of both CKs in the wound edge is visible: the CK14 green-signal is structured in the upper part (with respect to the white dotted line) while a signal featured by high background noise is observed at the lower part of the wound edges. CK10-positive cells are differentiated keratinocytes of the remaining tissue near to the wound bed. CK14-positive cells filled 100% of the wound bed within 3 days but the epithelium formed by these keratinocytes appears non-organized. A high background noise of CK10 is observed which emphasizes the presence of basal keratinocytes in the wound bed. On day 8, keratinocytes stratification in different layers is better and clearly observed: signal of CK14 is well organized and is mainly localized in the basal layer of the multilayer epithelium. CK10 begins to be expressed appearing in the upper layers and it is directly related to keratinocytes differentiation.

FN expression was investigated as biomarker involved in the remodelling phase in the FT-skin wound model (Fig. 3).

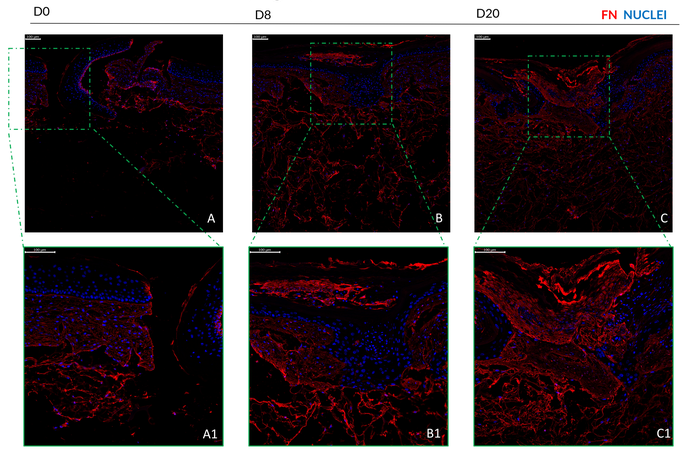

Fig. 3 IF of FN in the newly formed tissue of the wounded bed at Day 0, Day 8 and Day 20. Nuclei are stained with DAPI (blue). Magnification 10X and 20X. Scale bar: 100 µm.

At day 0, baseline expression of FN is visible in the extracellular matrix (ECM). Starting from day 8, in the wound bed, the fibroblasts increase the synthesis and deposition of FN with the aim of composing the granulation tissue (B and B1). Granulation tissue is formed during the proliferative phase of wound healing and it is composed by FN (mainly), collagen III, collagen I, hyaluronic acid and other proteoglycans that act as substrate for the epithelial cells migration. In our experimental conditions the proliferative phase continues from day 8 to day 20, increasing the deposition of FN in the wound bed (C and C1). FN quantification evaluated taking into account the intensity of the fluorescent signal that increases by 140% and 160% compared to day 0 at day 8 and day 20, respectively. Furthermore, from day 8 to day 20 it is possible to observe its expression in the cytoplasmic compartment: this uncommon expression seems to be related to boost the deposition of granulation tissue, inducing the wound closure.

CONCLUSION

We have demonstrated that, by using standardized injury procedure on 3D reconstructed FT-skin, it is possible to recapitulate the main steps of the skin regeneration and healing process in the two skin compartments (epidermis and dermis).
The described preclinical *in vitro* approach on FT-skin model has interesting applications:

- To assess the wound healing efficacy of dermo-pharmaceutical and cosmetic formulations at doses and exposures mirroring real life use
- To investigate at molecular level the specific mechanism of action of new ingredients on the different steps of wound healing process.

References:
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