Psoriasis Modeling with an Advanced Immunocompetent in Vitro System

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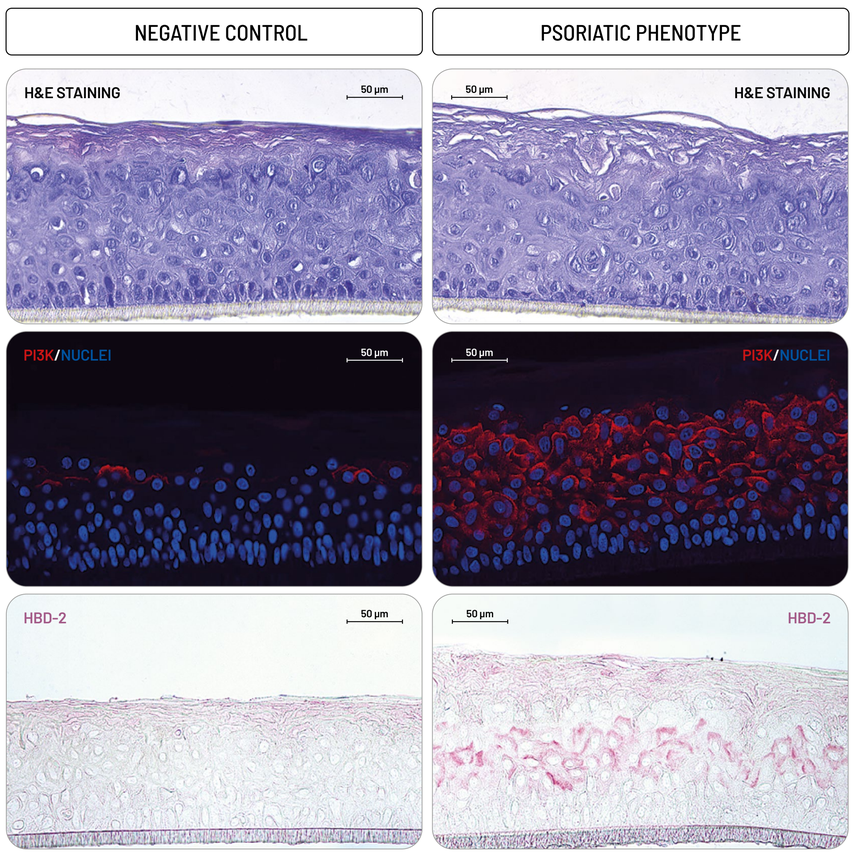
INTRODUCTION

Psoriasis is a chronic inflammatory skin disorder characterized by hyperproliferation and aberrant differentiation of keratinocytes, inflammation in dermis and epidermis and leukocyte infiltration by a Th1-like cytokine pattern. As part of our R&D activities a simplified *in vitro* model of psoriatic skin on Reconstructed human Epidermis (RHE) cultivated with Th1 cytokine mix (IL-17, IL-22, TNFα 1,5 ng/mL) has been first developed and the model has been characterized by an HIC approach **Fig.1 - Psoriatic Phenotype: Simplified Model On Rhe**



Morphological modifications compared to negative control RHE:

- **acanthosis,** characterized by an increase in the cells that make up the spinous layer (thickening of the basal layer, which consequently feeds the spinous layer)
- **hypogranulosis,** characterized by a reduction in the number of cells in the granular layer of the skin, resulting in a decrease in keratohyalin granules.

PI3K protein and HBD-2 were overexpressed suggesting the onset of inflammation, abnormal cell proliferation and involvement of innate immunity dysregulation characteristic of psoriatic lesions.

EXPERIMENTAL DESIGN ON 3C SYSTEM

Based on these preliminary findings **an advanced disease model** to better recapitulate psoriasis feature has been developed by using a multi-compartment human reconstructed full-thickness skin (3C System) co-cultured in the dermal compartment with THP-1 cells.
The same Th-1 cytokine mix known to be relevant for psoriasis pathology [1] was used to emulate the inflammatory stress. In order to validate the adavanced 3C system the keratinocytes response was evaluated at mRNA level by qRT-PCR using two dermatological formulations for psoriasis treatment:

- **Product A,** cream, 0,1% mometasone furoate, as steroid that mimics the actions of naturally occurring corticosteroid.
- **Product B,** emulsion, containing 50µg/g calcipotriol + betamethasone 0,5 mg/g as vitamin D derivative +anti-inflammatory compound.

Thee results wer compared to negative control (**NC**, untreated test system) and positive (**PC**, control exposed to cytokine mix and co-cultured with THP-1) controls. All the serie were evaluated at 2 time points:
- **TIME POINT 1:** 48H
- **TIME POINT 2:** 48H+24H

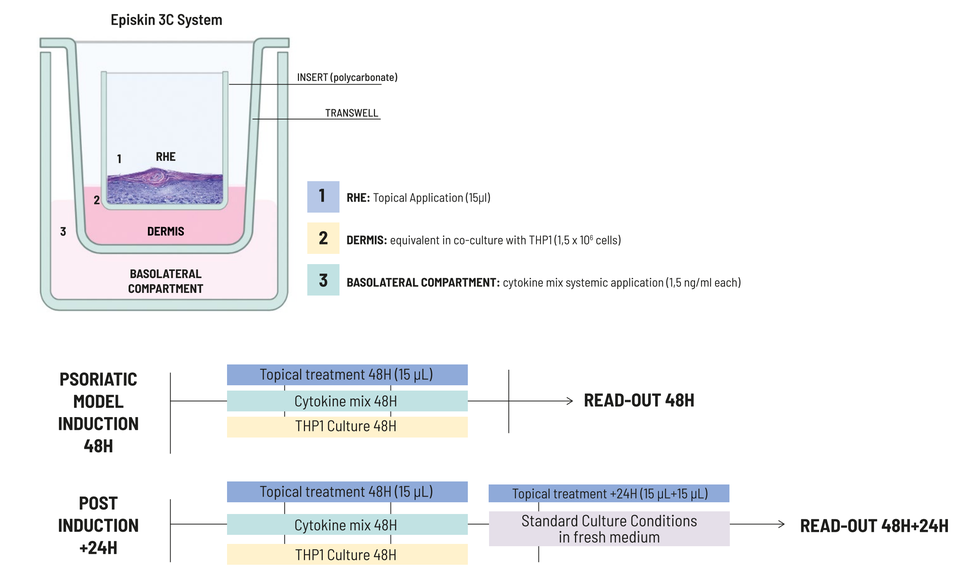


Figure 2: Experimental Design

The 3Csystem model (EpiSkin SA) is made up of 3 compartments:
1. Compartment containing **RHE** in air-lift culture and in contact with the dermis.
2. **Dermis** equivalent placed in a transwell made up of fibroblasts, collagen (mostly present collagen types IV, VII and XII), Laminin V, BP antigen.
3. **Culture medium**, used for model maintenance.

3Csystem model was cultivated in Episkin growth medium.
THP-1 cell line (Human Myeloid Leukemia cell line, HTL97014, ICLC) was cultivated up to the required density with RPMI1640 (EuroClone S.p.a) with the addition of 10% FBS, 1% P/S, 1% L-glutamine (2mM).
Reconstructed Human Epidermis (RHE) (Episkin SA) was systemically exposed to Th-1 cytokines mix for 48h, and co-cultured with 0,25mL of THP-1 (1,5x10^6 cells/in their medium) in the dermal compartment. Cytokine mix was prepared in EpiSkin maintenance medium. The psoriatic model was then treated topically with 15µL items directly on epidermal surface for 48h (time point 1) and 48h+24h (time point2).

Target genes investigated by qRT-PCR are listed in **Tab. 1**.

GENES	GENE ID	BIOLOGICAL ROLE
PSORIASIS	S100 A7 S100 A8 S100 A9	Facilitating leukocyte arachidonic acid trafficking and metabolism, modulation of the tubulin-dependent cytoskeleton during migration of phagocytes
		S100 A9 can induce neutrophil chemotaxis, adhesion, can increase the bactericidal activity of neutrophils by promoting phagocytosis via activation of SYK, PI3K/AKT, and ERK1/2
	PI3	PI3K is involved in several key signaling psoriasis pathways that are relevant to psoriasis, including those mediated by various cytokines and growth factors leading to increased proliferation of skin cells (keratinocytes) and the recruitment of immunocompetent cells
	SERPINB4	SERPINB4 modulate the activity of proteases, which are enzymes that can degrade other proteins and are often involved in psoriasis inflammatory responses
BARRIER FUNCTION	CLDN1	CLDN1 is required to prevent the paracellular diffusion of small molecules through tight junctions in the epidermis and is required for the normal barrier function of the skin. Required for normal water homeostasis and to prevent excessive water loss through the skin
	CLDN8	Mediates recruitment of CLDN4 to tight junction in the kidney
	CLDN23	Plays a major role in tight junction-specific obliteration of the intercellular space, through calcium-independent cell-adhesion activity
	FA2H	Responsible for the synthesis of sphingolipids and glycosphingolipids involved in the formation of epidermal lamellar bodies critical for skin permeability barrier
	K16 (alias KRT16)	Acts as a regulator of innate immunity in response to skin barrier breach: required for some inflammatory checkpoint for the skin barrier maintenance

RTqPCR: RNA extraction - retrotranscription and data acquisition
RNA extraction of the nucleic acids was performed using RNAqueous kit (Ambion, Life Technologies). RNA was eluted in RNAse free water, quantified by spectrophotometric assay, and stored at -80°C until use. The High-Capacity cDNA Reverse Transcription kit (Ambion, Life Technologies) synthesized cDNA from RNA, and gene expression was studied using Applied Biosystems 7500 Fast Real Time PCR (Thermo Fisher Scientific) with TaqMan assays, enabling Fluorescence data from the ABI 7500 Fast thermocycler are processed using SDS 2.0.6 software, and Raw Data, including gene expression information, is exported to Excel, with significance determined by when gene expression is at least 2-fold upregulated (RQ>2) or downregulated (RQ<0.5) compared to the calibrator sample (95% confidence level).

RESULTS

Gene expression by qRT-PCR

NC = 1 at defined time points		RQ	
		TIME POINT 1	TIME POINT 2
		PC	
PSORIASIS	S100A8	6,73	5,13
	S100A9	7,05	6,46
	PI3	8,94	5,59
	S100A7	37,85	18,77
	SERPINB4	9,23	1,37
BARRIER FUNCTION	CLDN1	0,75	0,72
	CLDN23	0,56	0,54
	CLDN8	0,58	0,45
	FA2H	0,76	1,27
	KRT16	2,27	3,06

Figure 3: Psoriatic Phenotype

Positive Control: TH-1 psoriatic mix has induced:
- Stable up-regulation of psoriatic genes PI3, S100A7 as expected; SERPINB4 24h after model induction returned to basal level.
- Up-regulation of S100 family genes S100A8 and S100A9, and for KRT16
- Down-regulation of Claudin family genes CLDN23 and CLDN8

Up - regulated
Down - regulated

NC = 1 at defined time points		RQ			
		TIME POINT 1		TIME POINT 2	
		Prod. A	Prod. B	Prod. A	Prod. B
PSORIASIS	S100A8	5,37	2,44	4,45	1,27
	S100A9	6,44	3,17	8,86	2,89
	PI3	7,14	4,21	5,48	2,76
	S100A7	25,78	10,84	7,55	3,14
	SERPINB4	13,16	5,25	1,46	1,86
BARRIER FUNCTION	CLDN1	0,77	0,65	2,22	2,86
	CLDN23	1,77	0,93	7,42	7,76
	CLDN8	1,17	0,63	2,41	2,64
	FA2H	0,52	0,26	0,44	1,01
	KRT16	2,23	1,88	8,93	6,22

Figure 4: Topical Treatments Results

PRODUCT A: the dermatological product containing 0,1% mometasone furoate, compared to psoriatic control, has counteracted the psoriatic transcriptional profile and stabilized the genes involved in barrier integrity:
- Up-regulation of Claudin family genes (CLDN23 and CLDN8);
- Down-regulation of S100 family genes (S100A7 and S100A8).

PRODUCT B: the dermatological product containing 50mg/g calcipotriol + betamethasone 0,5 mg/g has shown efficacy in counteracting the psoriatic transcriptional profile compared to psoriatic control:
- Up-regulation of CLDN23 and CLDN8 (over the basal levels at TIME POINT 2)
- Down-regulation of S100 family genes (S100A7, S100A8, S100A9)
- Down-regulation of all genes for psoriasis PI3,S100A7 and SERPINB4.

Efficacy in counteracting psoriatic expression pattern

CONCLUSION

- The biological relevance of the new 3C System + THP1 co-culture as advanced psoriasis model is supported by the significant over expression of genes PI3, S100A7, and SEPINIB4 in response to a psoriasis-induction protocol following 48 hours of exposure to a Th-1 cytokine mix.
- In a post-induction experimental window (24 hours of recovery in standard medium), the psoriasis transcription profile was confirmed for PI3 and S100A7, while the gene expression of SERPINB4 had reverted to its baseline level.
- The exposure to the Th-1 psoriatic cytokines led to the up-regulation of other genes within the S100 family and KTR16, whereas members of the Claudin family exhibited down-regulation at each time point, albeit with varying degrees of magnitude. These findings are in agreement with existing literature [1] [2] [3].
- The maintenance of a psoriasis-like transcription profile has a wide of experimental window up to 72h: indicates that the model can be used to test product efficacy in post-induction application protocols.
- Both dermatological anti.psoriatic formulations have shown an efficacy to counteract the psoriatic transcriptional profile, albeit with varying degrees of efficacy (PRODUCT B > PRODUCT A) suggesting that the proposed in vitro advancd psoriatic system is a predictive biological model responsive to topical treatments: it can be applied to differentiate product efficacy and explore their mechanisms of action.

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