Development of an original reconstructed tissue model reproducing nipple epithelium to evaluate anti-inflammatory and repairing efficacy of a breastfeeding balm

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Introduction

Reconstructed human skin tissues provide useful test systems to set-up experimental models in order to evaluate cosmetic products efficacy. Indeed those models are highly reproducible, predictive at both cellular and molecular levels, and display functionality close to the *in vivo* mechanisms.

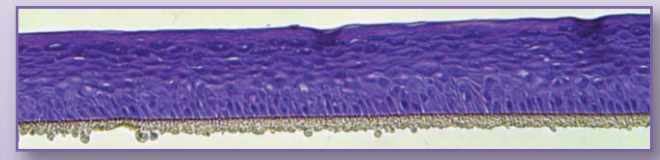
There is a great need for the development of tissue models mimicking the specific characteristics of the skin in certain conditions. For example, the nipple epithelium is considered to be a specialized epidermis that shows distinct patterns of differentiation and keratin's expression to withstand the mechanical strength of nursing.

Based on the knowledge of those particular features of the nipple epidermis, we have developed and characterized a specific nipple-tissue reconstructed model. This tissue was deeply injured in order to mimic fissures and cracks which may occur in breastfeeding. We used this nipple-tissue model to assess the soothing and repairing efficacy of a nursing balm.

SET UP AND CHARACTERIZATION OF A PATENTED NIPPLE EPIDERMIS MODEL

Histologically, nipple epidermis appears thickened and hyperkeratotic with expanded suprabasal, granular and cornified layers. At the molecular level, it is characterized by an increased level and specific distribution of filaggrin and keratin 14, among others. These features influence the barrier function properties and lead to different tissue response to external stimuli.

In order to develop a nipple epidermis model, reconstructed epidermises have been produced under various culture conditions and from different human keratinocytes strains, selected for their high filaggrin production capacity.
Comparative histochemical and immunohistochemical analysis allowed us to choose the conditions reproducing the better the histological features of nipple epithelium:
We selected the keratinocyte strain and culture conditions that yielded to epidermis overexpressing filaggrin and presenting a high number of granular layers and numerous keratohyalin granules.



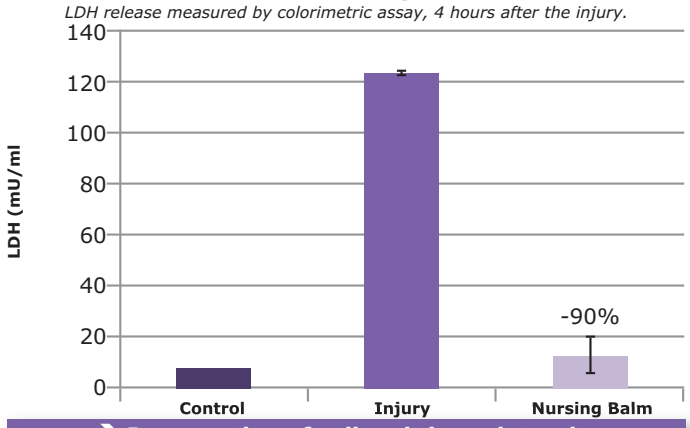
Patented reconstructed nipple epidermis (D17)

Use of the nipple model to evaluate a breastfeeding balm

Methods
The reconstructed nipple epidermis model was wounded: a mechanical injury was realized on the surface, to mimic a fissure or crack.
After what, the assayed product was topically applied and the epidermis incubated for 4 hours or 24 hours.

LDH RELEASE

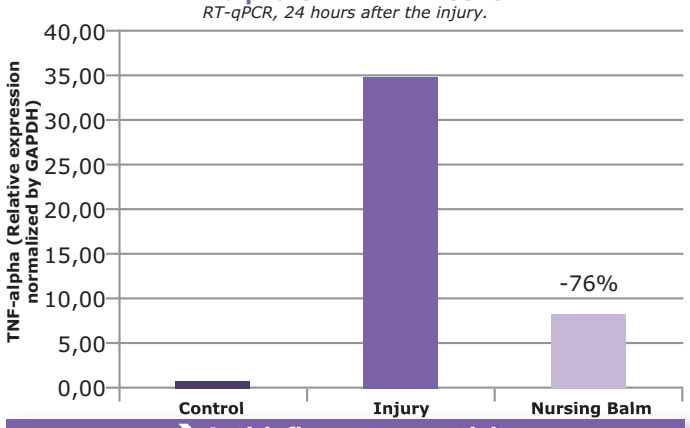
LDH release measured by colorimetric assay, 4 hours after the injury.



➔ **Preservation of cell and tissue integrity**

TNF-alpha GENE EXPRESSION

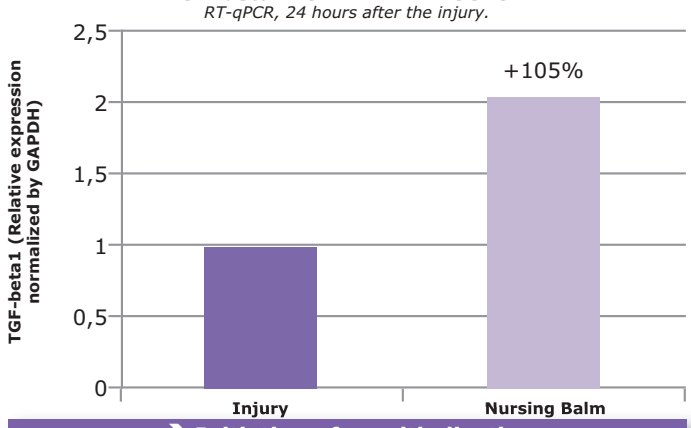
RT-qPCR, 24 hours after the injury.



➔ **Anti-inflammatory activity**

TGF-beta 1 GENE EXPRESSION

RT-qPCR, 24 hours after the injury.



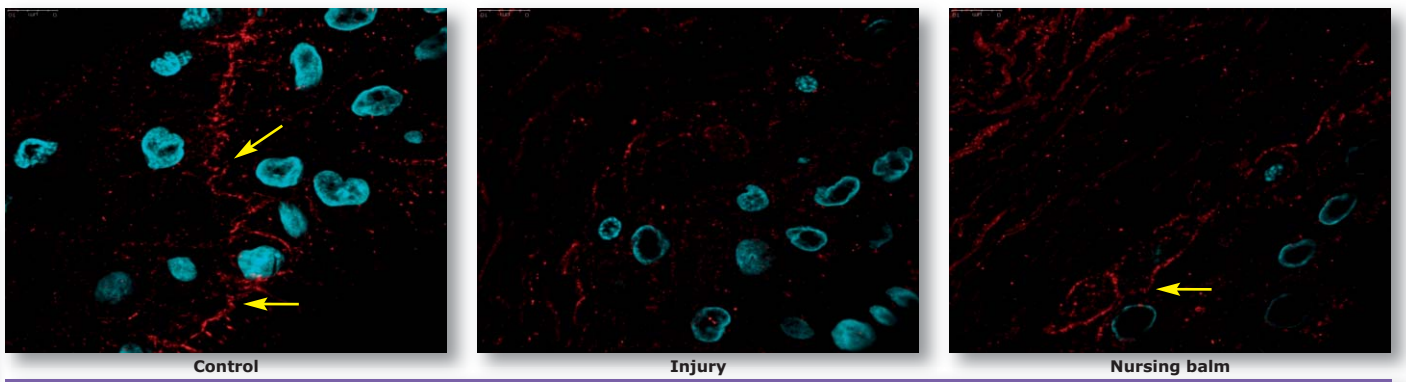
➔ **Initiation of reepithelization**

The injury of the nipple epidermis model reproduced the fissures or cracks that may occur during breastfeeding. This integrated model unables to study the deleterious effects of fissures at the cellular and molecular level.

Topical application of the nursing balm after wounding of the nipple epidermis was able to protect from cell damage and to preserve tissue integrity, as well as to modulate the inflammatory signal that contributes to the pain sensation. Moreover the balm showed strong repairing and protective properties.

INTEGRIN-beta 1 EXPRESSION

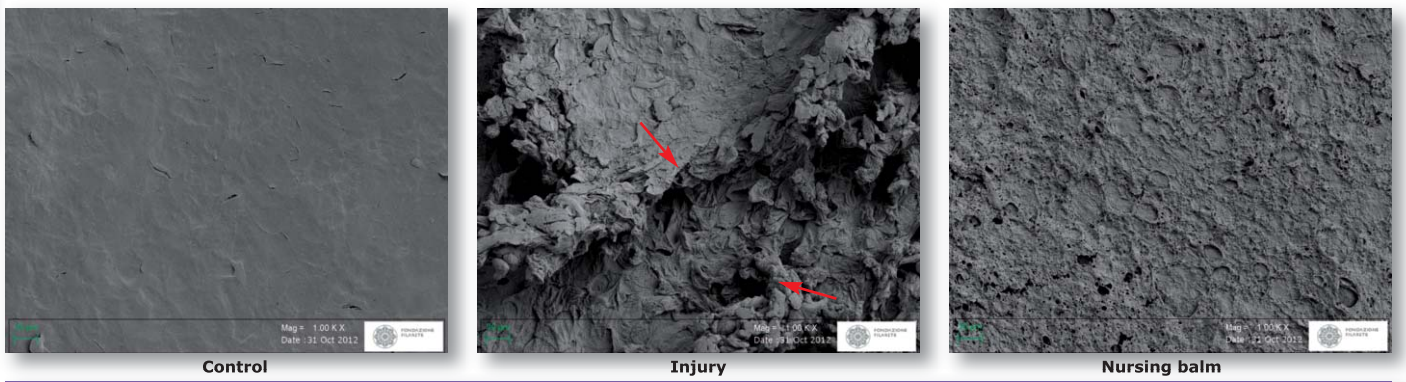
Immunostaining of beta 1-integrin revealed by AlexaFluor555 (red), counterstaining of the nuclei by DAPI (blue); Confocal microscopy, magnification x63; 4 hours after the injury.



➔ **Initiation of healing process**

VISUALISATION OF THE MICRORELIEF

Scanning electron microscopy, magnification x1000; 4 hours after the injury.



➔ **Protective and repairing effect**

Conclusion

Basing ourselves on the bibliographic knowledge of the nipple epithelium physiological features, we have developed a specific nipple-tissue reconstructed model that was so far unavailable.

This original model allowed us to mimic painful fissures or cracks that may occur during breastfeeding and to study their consequences at the molecular level.

We used this model to show the protecting, soothing and repairing efficacy of a nursing balm.

This balm has also proved its efficacy *in vivo* during a clinical study (data not shown).