# New ex vivo model in wound care highlights a natural skin regenerating booster

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## Introduction:

Wound healing is a complex mechanism which is implemented by the perfect coordination of 4 phases: hemostasis, inflammation, proliferation, and remodeling (1). Excessive and prolonged inflammation promotes wound healing delay and excessive scaring (2). Thus, the switch between proinflammatory phase and skin reparative phase (proliferative and remodeling phases) is crucial.

We developed an annular skin explant model (ASEM) that mimics this delicate transition and thus screen effective products that may accelerate this process. In a first step, we investigated the natural repairing kinetics of our ASEM by analyzing epidermis and dermis markers at mRNA and protein levels. In a second step, a promising cream was applied on ASEM every two days for 14 days to evaluate its regenerating properties.

## Materials & Methods:

In this study, we collected a human skin explant obtained from a 55 years-old Caucasian female donor undergoing abdominoplasty surgery. We performed a double excisional skin wound in human skin explant using 10 mm and 2 mm punches to obtain our Annular Skin Explant Model (ASEM). It is then placed into a 12-well tissue culture dish with a solid nourishing fibrinogen matrix and dedicated medium. The figure 1 schematizes the method to obtain the ASEM. The cream was applied into the wound and around the wound on the explants for topical application. Explants were collected on day 0, 7 or 14 after punch dissection.

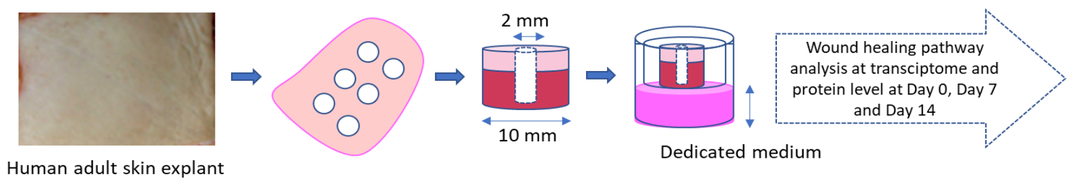


*Figure 1 Annular Skin Explant Model (ASEM)*

## Results & Discussions:

Wound healing begins by a hemostasis phase followed by an inflammation phase. The inflammation phase controls sequential infiltration of immune cells and cytokine release, including IL6 (3), which controls MMPs protease. MMP-3 controls matrix degradation but also regulates chemokine expression and activity by their degradation or the production of receptor antagonists (4). Then, the proliferative phase focuses on covering the wound surface by re-epithelialization, repairing the vascular network and reconstructing granulation tissue. MMPs, PCNA and COL III are thus expressed to manage this phase (5). Finally, the remodeling phase improves wound contraction by transforming fibroblasts into myofibroblasts which express ACTA2 (6). We also observe the restoration of the normal structure and function of the epidermis and dermis with the expression of epidermal markers of differentiation such as LOR, LAMA5, FLG,... (7).

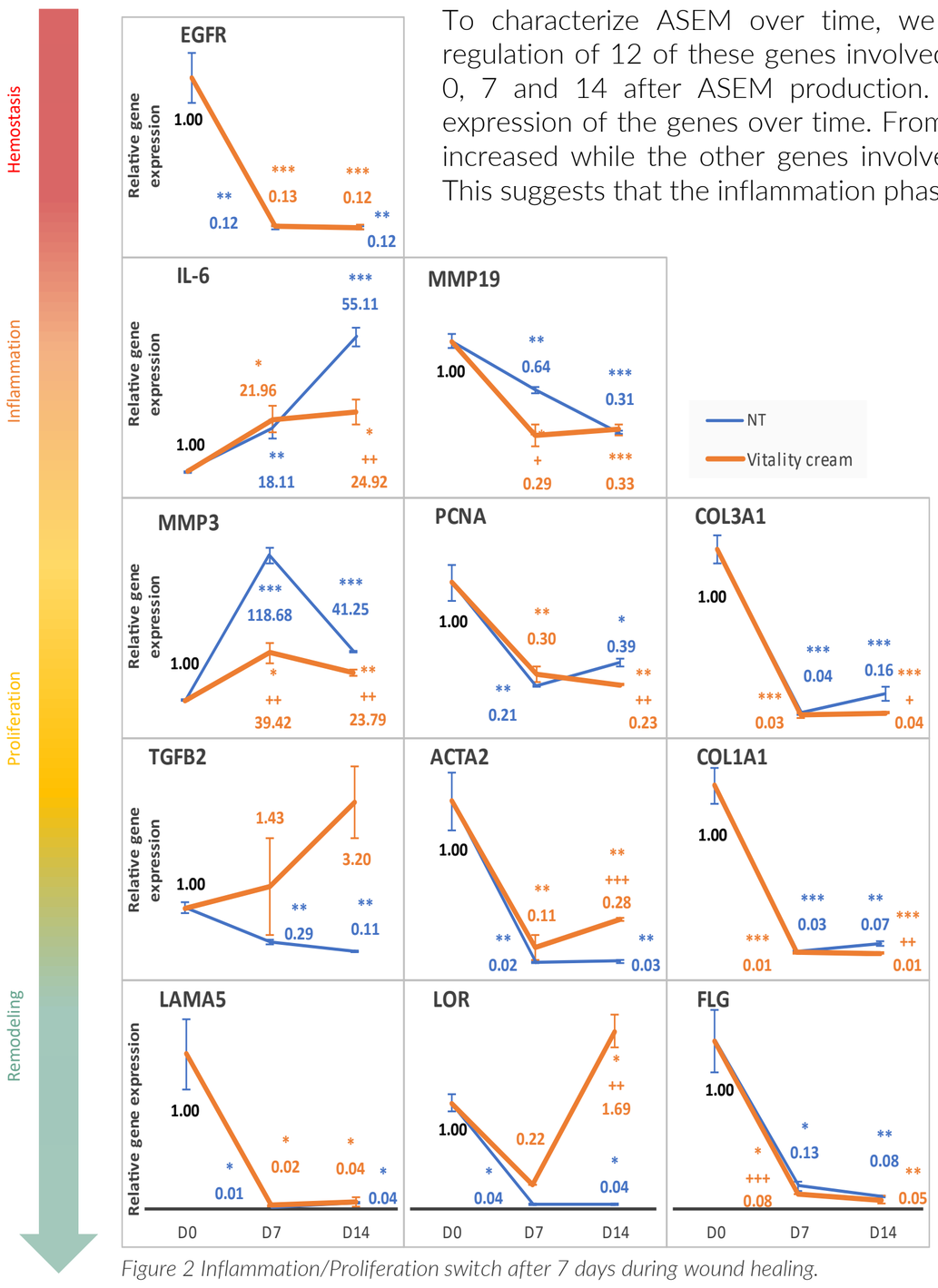


*Figure 2 Inflammation/Proliferation switch after 7 days during wound healing.*

To characterize ASEM over time, we firstly evaluated by quantitative PCR the gene expression regulation of 12 of these genes involved in inflammation, proliferation, and remodeling phases at days 0, 7 and 14 after ASEM production. The graphs presented in figure 2 showed the quantitative expression of the genes over time. From day 0 to day 7, the inflammatory genes MMP3 and IL6 were increased while the other genes involved in proliferation and remodeling were drastically decreased. This suggests that the inflammation phase occurred during this period.

From day 7 to day 14, the induction of IL6 and the repression of the others was maintained or accentuated while the induction of MMP3 is blocked. Moreover, proliferative phase regulators such as PCNA and COLs were up-regulated compared to day 7, suggesting the start of the proliferative phase between day 7 and day 14.

Taken together, our results highlighted that the Inflammation/proliferation switch seems to occur about 7 days after the wound. This observation is consistent with what happens in vivo. Indeed, previous authors observed that in a mouse model (C57BL/6J WT) the model enter in proliferative phase at day 7 after the wound (8). The MMP3 repression and PCNA induction seem to be key characteristics of this delicate transition.

To corroborate this gene regulation at protein level, we performed a MMP3 and PCNA immunohistochemistry staining (figure 3). Quantification of MMP3 positive areas in the dermis and PCNA positive areas in the epidermis showed consistent results with our mRNA expression.

Treatment with the vitality cream had no effect on FLG, LAMA5, EGFR gene regulation. However, from day 0 to day 7, this treatment accelerated MMP19 gene repression and reduced MMP3 induction at mRNA and protein level demonstrating a reduction of the inflammation phase and an earlier inflammation/proliferation switch. From day 7 to day 14, vitality cream treatment inhibited IL6, COLs genes expression and PCNA at mRNA and protein level while ACTA2 and LOR genes were increased. Clearly, from day 7 to day 14 the vitality cream seems to initiate the remodeling phase.

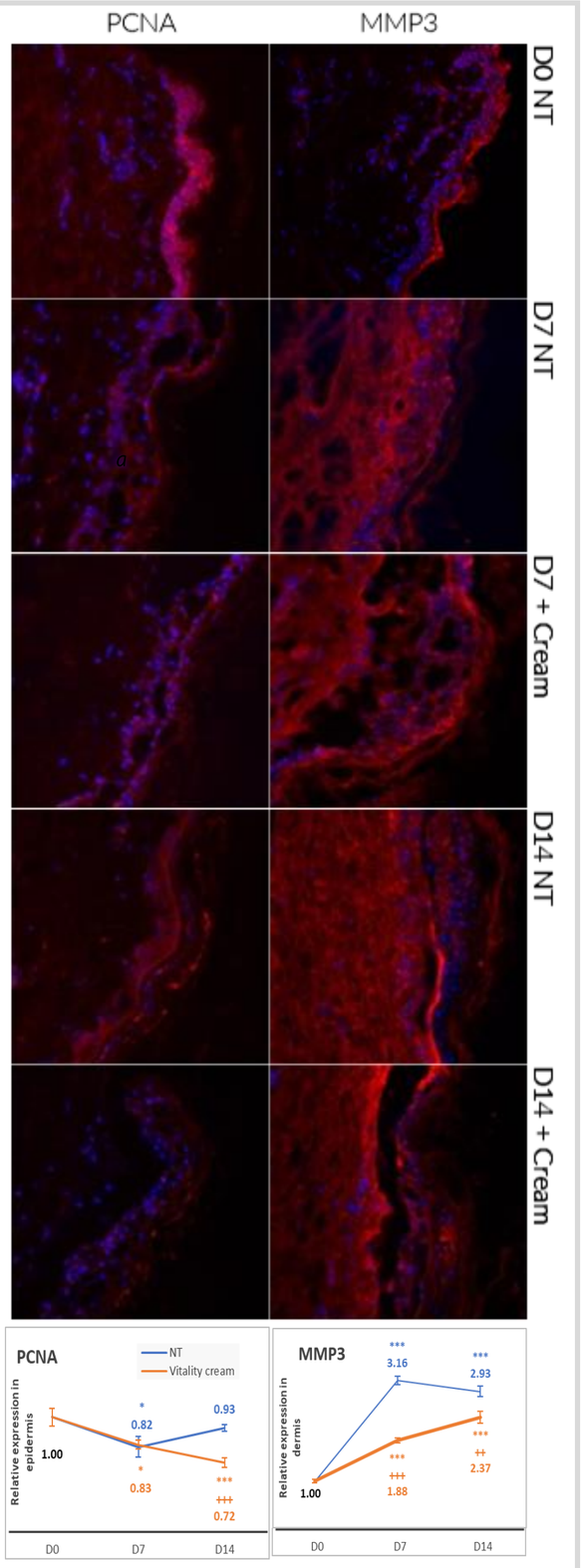


*Figure 3 MMP3 and PCNA IHC labelling. The mean fluorescence intensity was measured inside the dermis for MMP3 or the epidermis for PCNA . Pictures of the stained sections for each condition are shown .*

## Conclusions:

The figure 4 provides a schematic illustration of the mechanism of ASEM model overtime. Taken together our data showed that from day 0 to day 7, our ASEM is clearly in the inflammation phase with the over-expression of IL6 and MMP3 combined to the repression of the specific markers of the other phases such as keratinocytes differentiation markers. From day 7 to day 14, MMP3 induction is blocked, the expression of PCNA, a key proliferative inducer, and collagens start to increase.

In such context, the vitality cream treatment displays a promising regenerative activity by decreasing the inflammation duration, promoting the wound contraction and improving the remodeling phase.

ASEM model is of interest to highlight efficient cosmetic products able to accelerate inflammation and proliferative phase switch and avoid unsightly scars or chronic wounds that are difficult to cure.

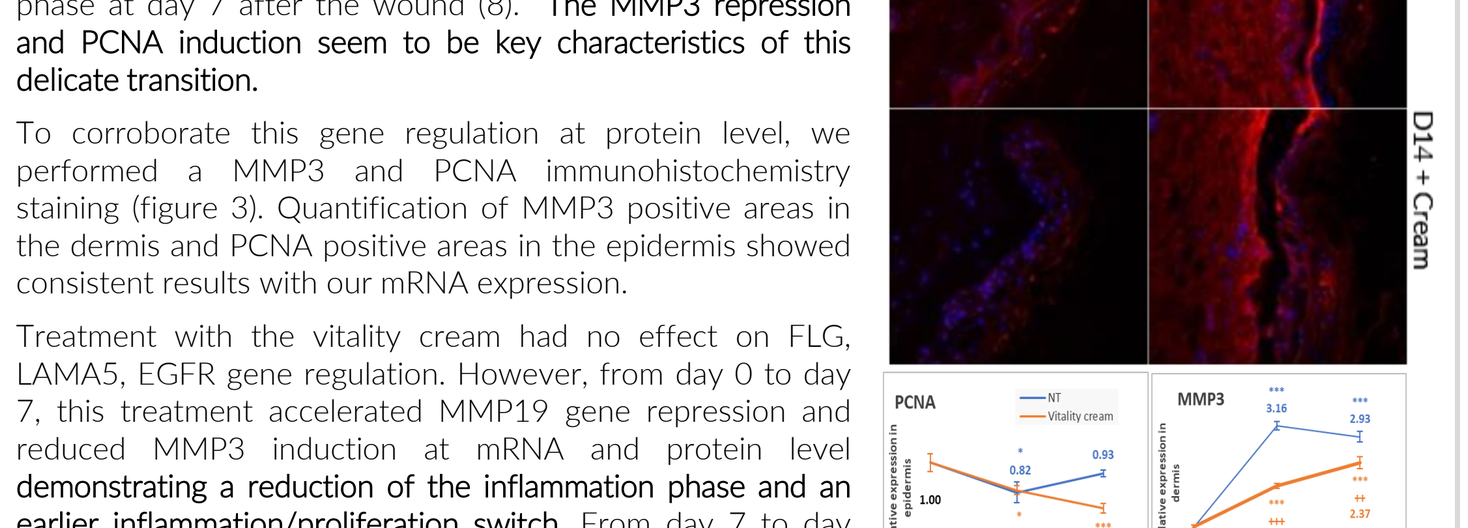


*Figure 4*
*Schematic description of the wound healing process. The four major phases are presented on the left: Hemostasis, Inflammation, Proliferation and Remodeling. The figure shows the progress of the wound healing process in the model, with or without cream application, after 7 and 14 days.*

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