Stimulation of the KLF4 pathway by bee products modulates the progression of hair anagen to telogen molecular switch.

Forraz, Nico1; Kurfürst, Robin2; Jeanneton, Olivier2; Desroches, Anne-Laure1; Payen, Pauline1; Milet, Clement1; Vialle, Alize1; Choisy, Patrick2; Nizard, Carine2; Pays, Karl2; McGuckin, Colin1.*
1 CTISkin department, CTIBIOTECH, Lyon-Meyzieu, France ; 2 LVMH Recherche, Saint Jean de Braye, France ;

Poster-ID : 182

INTRODUCTION

Medicines containing natural bee products are gaining more attention. Numerous studies have now proven the positive benefits of bee products for medical treatments but also as cosmetic ingredients. Each bee product possesses specific components which determine their activities. Skin biology has also linked the epidermal-hair axis as an active and effective route for ingredient interaction for regeneration.
Here we developed an accurate hair follicle – dermis – epidermis model to effectively evaluate hair phase kinetics through to promotion of coloration in the epidermis and hair itself. Several known molecules, receptors and signal transduction pathways have been identified as being key in the development and growth of hair and particularly hair phases of anagen, catagen and telogen.

METHODS

EX VIVO MODEL

Punches of 8mm² from human fresh scalp skin with high density of hair follicles (56-year-old, Caucasian, female). Samples placed in 12-well plates with culture medium for maintenance. Samples were placed into skin culture medium (CTIBiotech proprietary medium) for maintenance before the testing phase.

ACTIVE APPLICATION

Combination of four honeys and royal jelly forming the testing product in skin growth medium (CTIBiotech). The product was applied daily for 10 days using a sterile gauze to retain it on top of the biopsies and avoid any systemic application in the medium. No rinsing was carried out during time of culture before a new application of the product. Each condition was performed in triplicate.

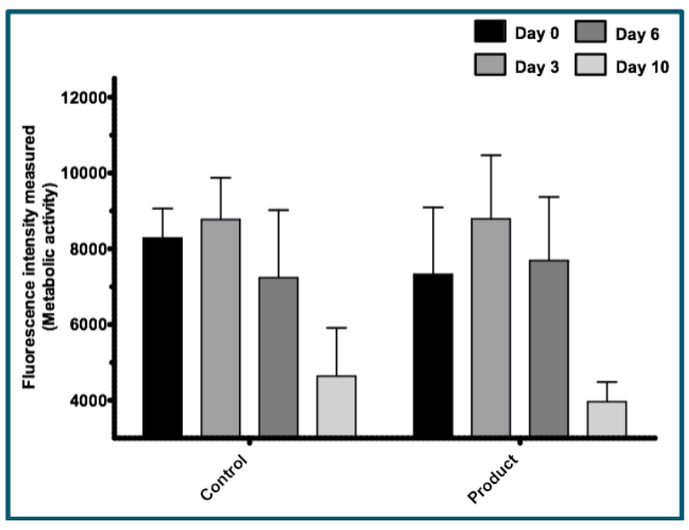
METABOLIC ACTIVITY

To assess metabolic activity, the AlarmarBlueTM Cell Viability reagent (Invitrogen) was directly added in each individual well and incubated for 4 hours. Supernatants were collected and transferred to a black-walled 96-well plate. Fluorescence was quantified on a microplate reader (Tecan Spark®) at 550 nm, excitation and 590 nm emission. This protocol was repeated on day 0, 3, 6 and 10 to follow metabolic activity kinetic.

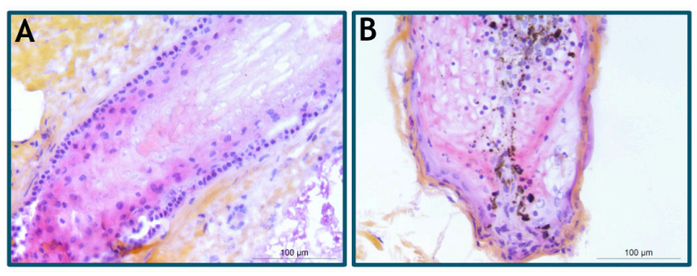
BIOLOGICAL IMAGING

Half of explants were rinsed and fixed, before dehydration in alcohol crescent baths and clarification in xylene. Samples were then embedded in paraffin and sectioned into 5 µm thick slices. Hematoxylin, eosin and saffron (HES) coloration were performed.
Second part of explants were fixed in OCT and stained with antibodies against FGF9, IGF-1, FGF5, KLF4, FGF7, TGF-β, Versican and KGFR receptors. A staining of nuclei was also performed using DAPI. To maximise data, staining of samples was grouped where possible to hair phase unless the antibody had cross reactivity. Acquisition was carried out using Leica DMLB Fluorescence microscopy.

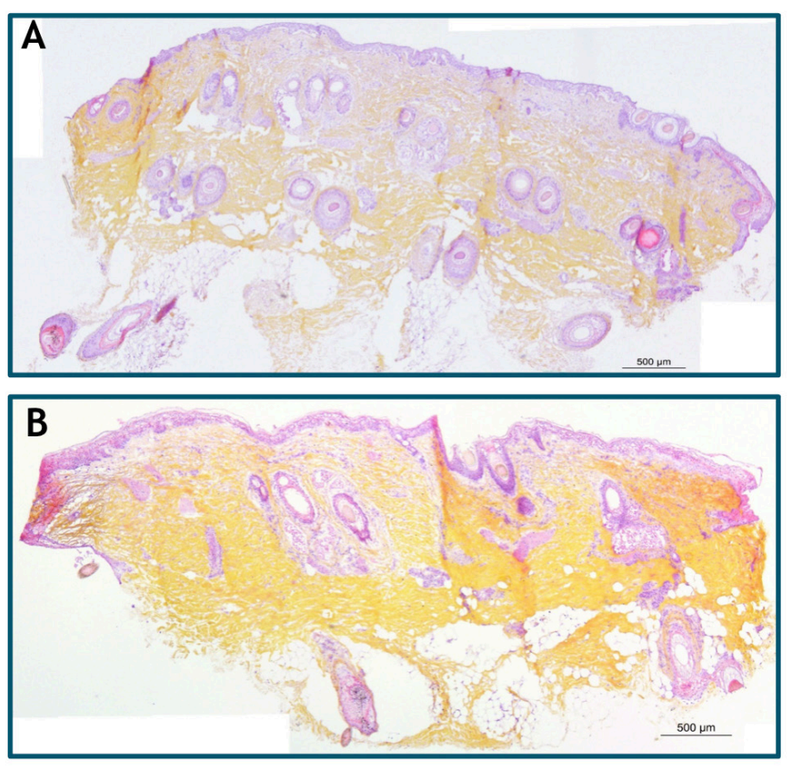
RESULTS



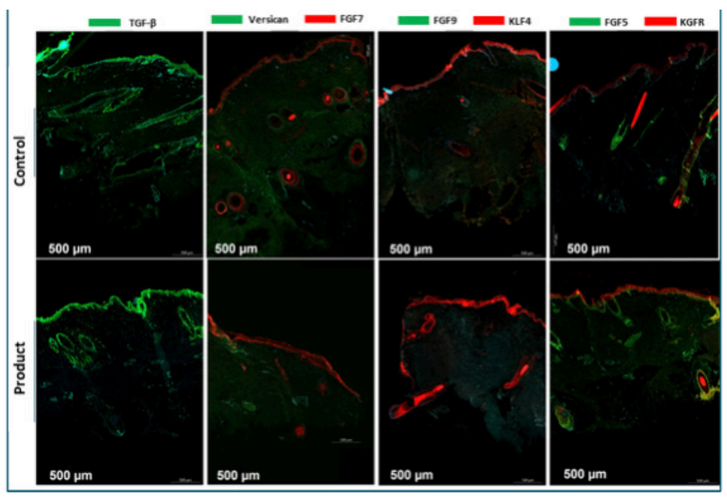
- No difference in metabolic activity of the samples was observed between conditions



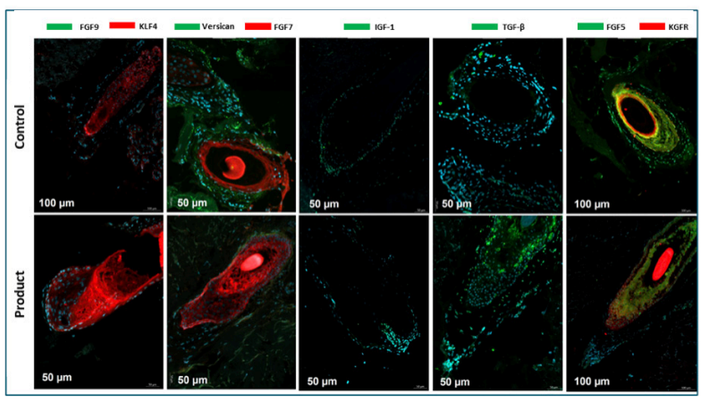
- Follicular analysis (HES staining) following active ingredient treatment (B) revealed upregulation of melanin expression.



- Full scalp histology revealed that active ingredient (B) increased cohesion of epidermis and dermal matrices.



- TGF β, FGF7, KLF4 and KGFR increased in treated samples.
  Higher level of differentiation-maturation cycling and turnover of keratinocytes : a thicker more proliferative epidermis.



- Promotion of hair bulb stem : KLF4 elevation.
  Anagen phase was promoted in the hair bulb : FGF7 and Versican were increased. / IGF-1was : increase. / TGF- β and FGF : lower, move away from telogen phase.

CONCLUSION

- Positive hydration effects associated to bee products application.
- Promoting effect on :
  - Epidermal keratinocyte kinetics
  - Melanin production in the hair follicle
  - Exert a stimulation of the stem cell compartment of the hair bulb.
- Good candidates cosmetic industry formulations dedicated to scalp and hair care.