KERASTIM® S

Ref. AC096

Pantin, February 23, 2009

1. In vitro effect on keratinocytes energy metabolism increase

a. Cytotoxicity study

It is required to carry out a cytological and toxicological test to specify the concentration of **KERASTIM® S** which will be used in cell cultures.

Non cytotoxic **KERASTIM® S** rate is 0.25%.

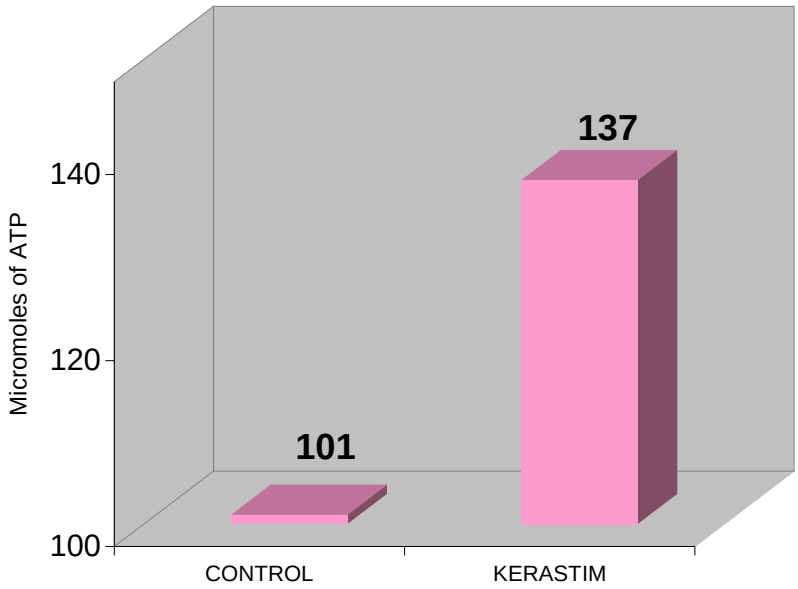
b. In vitro test

KERASTIM® S' ability to boost keratinocytes energy metabolism has been measured according to the production of intracellular ATP (ATP is a very good gauge for cellular metabolism).

Protocol

- Human epidermal cell culture
- Incubation of two cell ranges
  - TEST : presence of 0.25% **KERASTIM® S**
  - CONTROL : without test product
- After 4 hours contact, application of trypsine (to unstick cells of the culture box)
- Rinsing, centrifugation, cell lysis
- Measure of intracellular ATP level by bioluminescence (measure of photons produced during the reaction between the ATP present and D-luciferin in presence of magnesium oxide and luciferase).

Results



AFTER 4H CONTACT, KERASTIM® S INDUCES A + 35% INCREASE OF INTRACELLULAR ATP ON KERATINOCYTES

2. In vivo effect of KERASTIM® S on nail growth

a. Principle

The aim of the study is to highlight and measure the impact of **KERASTIM® S** on nail growth after a 30 day treatment.

b. Protocol

KERASTIM® S has been tested in a 5% emulsion on a two daily basis applications during one month on six female volunteers.

- D0

- delimit a surface area with two anatomic marks on two nails (one treated and one untreated)
- mark on nails of each hand
- image visualization and acquisition by video microscope of studied nails

- D0 - D30

- two daily applications

- D30

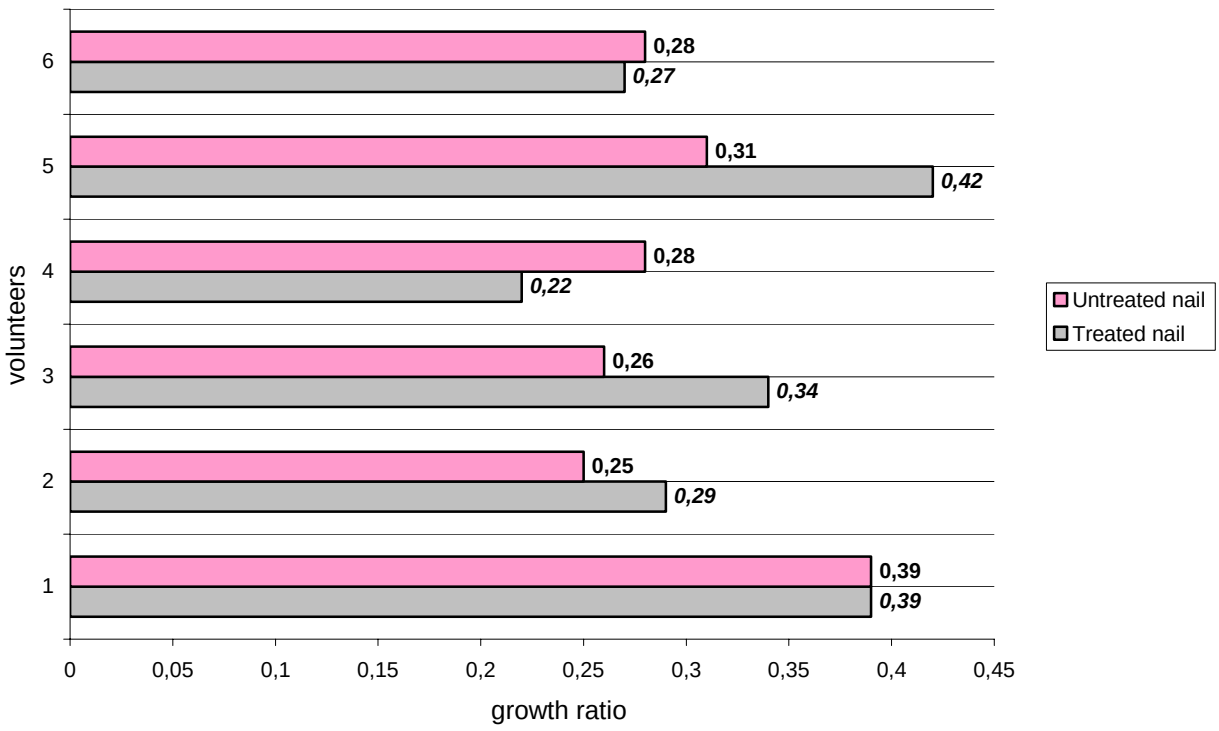
- spotting on the mark made on the nail at D0,
- image visualization and acquisition of studied nails by video microscope

c. Results

According to each volunteer, a growth ratio of treated (Coef Tr) and untreated nail (Coef NT) is set by image analysis.

Growth variation is written in percentage and calculated according to the formula :

$$\frac{(\text{Coef Tr} - \text{Coef NT}) \times 100}{\text{Coef NT}}$$



AFTER 1 MONTH TREATMENT, GROWTH OF TREATED NAIL WITH KERASTIM® S (5%) HAS INCREASED BY 26% COMPARED TO NON TREATED NAIL GROWTH IN 50% OF THE CASES

KERASTIM® S improves nail's beauty and quality :

- **Smooth surface**
- **Less brittle nail**
- **Less splitting nail**

3. In vivo anti hair loss effect of KERASTIM® S

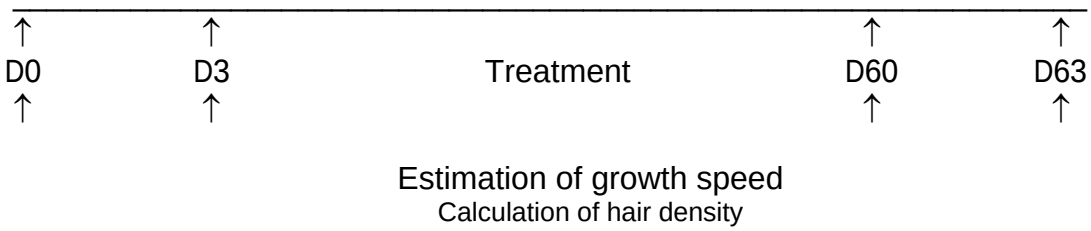
The aim of the study is to highlight and measure the anti hair loss impact of **KERASTIM® S** after a 60 day treatment.

KERASTIM® S has been tested at a rate of 5% in aqueous solution at a two months daily application on 6 volunteers having a chronic hair loss.

Measures have been taken under an optical video microscope connected to a computer system of image acquisition. Then, the image has been analysed by specific software which enable to measure hair density.

The phototrichogram distinguishes hair being at the anagen stage and hair being at the telogen stage.

Protocol



- A D0

- delimit a 25 mm^2 area on volunteer's hair scalp
- hair shave on this area
- macrophoto of the area under a video microscope, and computer saving to calculate the hair density

- D3

- macrophoto of studied area with computer saving
- calculation of hair density (anagen and telogen stages)

- D3 - D60 : treatment

- daily application of **KERASTIM® S**

- D60 : end of treatment

- hair shave on the studied area and new image recording
- calculation of hair density

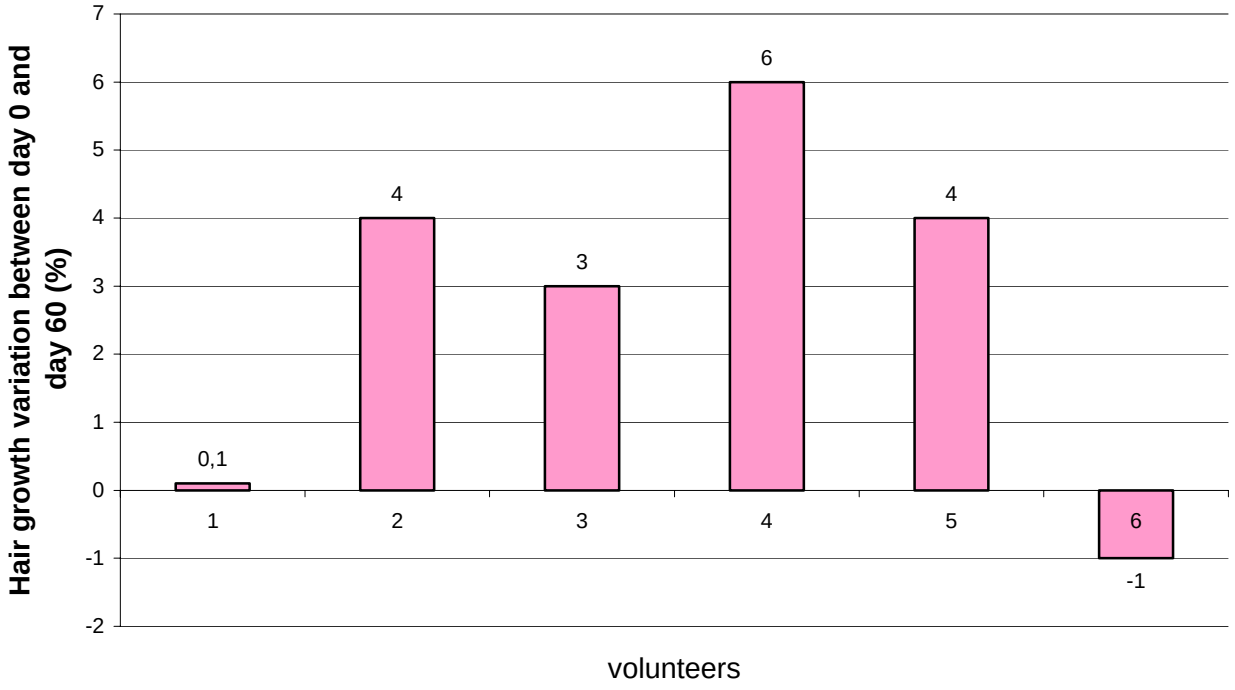
- D63

- macrophoto of the studied area
- calculation of hair density

Results

DEFINITION OF AN ANTI HAIR LOSS EFFECT

An active ingredient has a anti hair loss impact when the hair density after treatment is at least at the same level as before treatment on the studied area.



A 5%, KERASTIM® S :

- STOPS HAIR LOSS IN 82% OF THE CASES
- AVERAGE INCREASE HAIR DENSITY BY 3%