

# Evaluation of soothing activity of *Aphloia theiformis* extract using an innovative model of human innervated reconstructed epidermis

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# Introduction

**Sensitive skin** is defined by the occurrence of unpleasant sensations such as tingling, burning, itching or pain. Many hypotheses have been proposed to explain mechanisms involved in sensitive skin.

Because sensitive skin is primarily characterized by a wide variety of **neuropathic-like symptoms**, it is highly likely that neurosensory dysfunction in the skin represents one of the pathological mechanisms of sensitive skin. Especially, cutaneous innervation and excessive release of neuropeptides are recognized as major elements of skin physiopathology associated with neurogenic inflammation.

## Objectives

- develop and validate an innovative model of fully human innervated reconstructed epidermis
- determine if ATE extract is able to reduce neuropeptide release using this model.

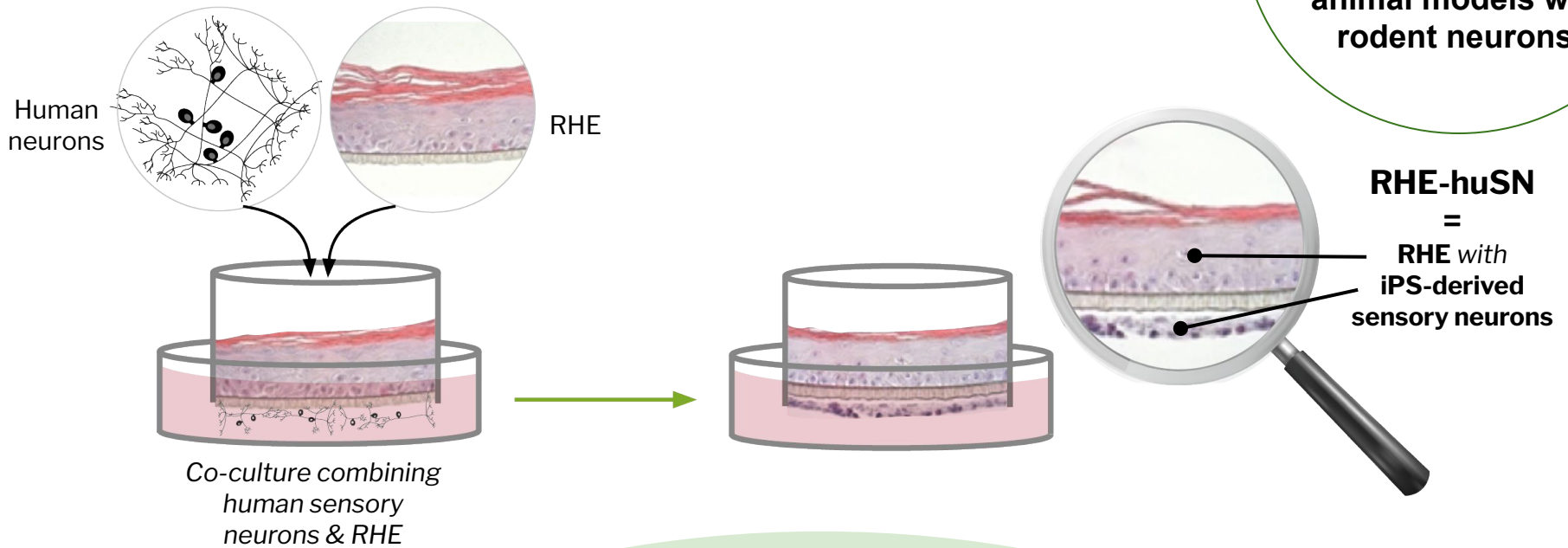


***Aphloia theiformis*** leaves traditionally used to treat fevers, pain, malaria and inflammation. The bark also used as emetic and the leaves as antipyretic.

→ Thanks to its **anti-inflammatory properties**, ATE extract isolated from *Aphloia theiformis* leaves, could provide a new target for the treatment and prevention of sensitive skin.

# An innovative 3D model

## The concept



Few experimental approaches available to evaluate neurogenic inflammation. Most of them rely on **animal models with rodent neurons.**

Reconstituted epidermis cultivated on inserts equipped with a porous filter with a seeding of human sensory neurons derived from hiPS on the lower face of the filter (**RHE-huSN**).

⇒ **a new model 3D** to evaluate the efficacy of actives or formulations to reduce neuropeptide release.

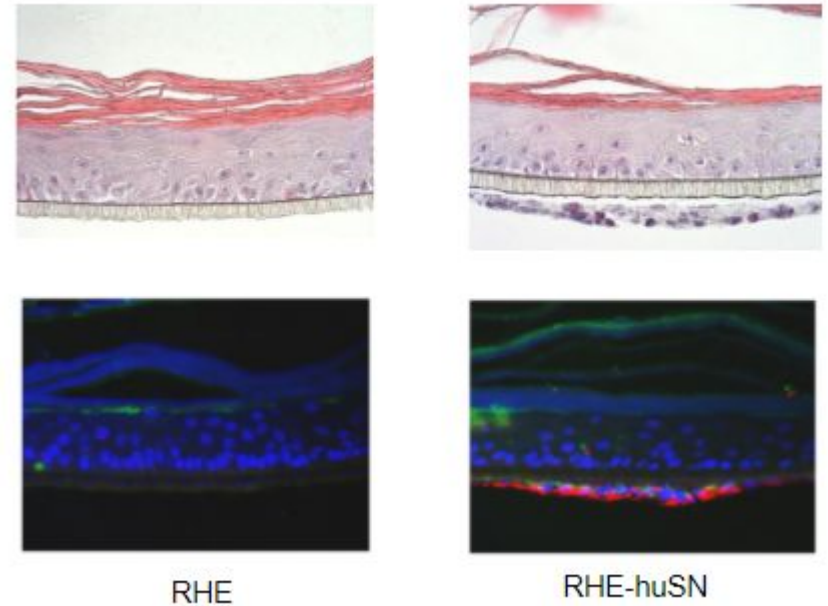
# Results

## A fully human innervated reconstituted epidermis

### Histological analysis and immuno-labelling

➤ Histological hematoxylin-eosin stainings: **morphology and cellular organization** similar to RHE tissues grown in the absence of neurons.

➤ Immuno-labelling ( $\beta$ -tubulin and neurofilament 200): **neurons** present on the lower face of RHE-huSN model are **not altered** in any of the tested conditions

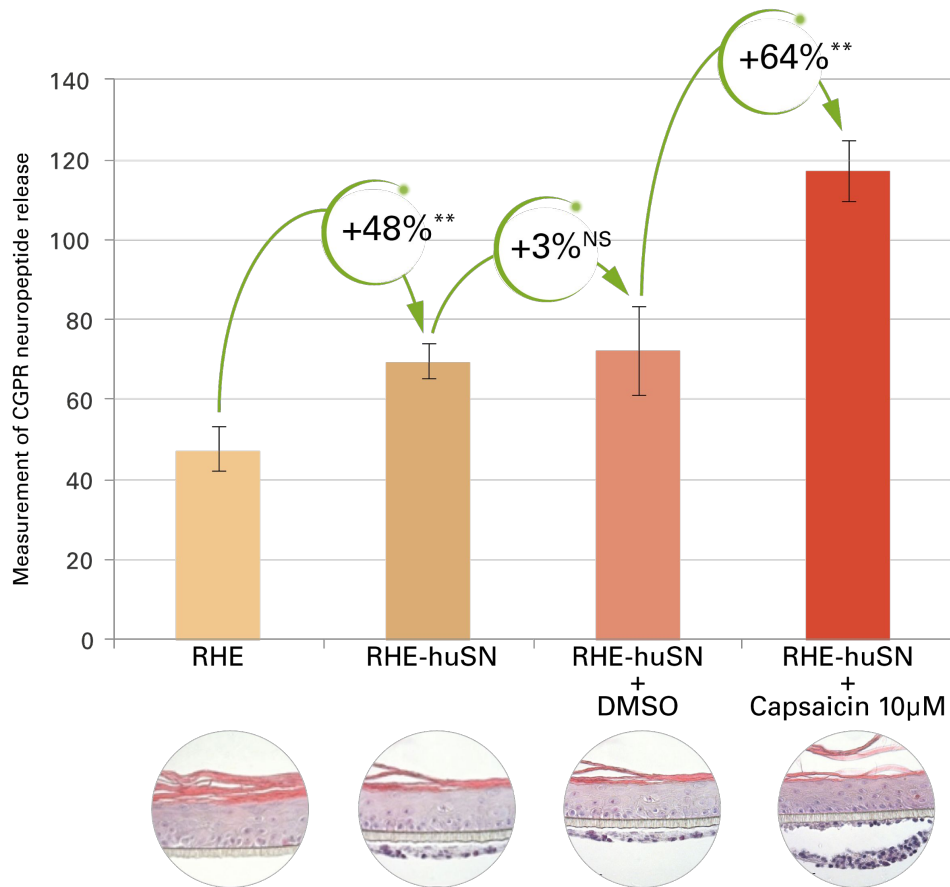


Histological analysis (hemalum/eosin staining) and immuno-labelling (green:  $\beta$ -tubulin, blue: DAPI, red: NF-20) of RHE-huSN

# Results

Student's test  
NS : not  
significant  
\*\*p<0.01

## A fully human innervated reconstituted epidermis



DMSO: capsaicin solvent

### CGRP release

➤ The activation of the RHE-huSN with capsaicin results in **an increase of the neuropeptide CGRP release** compared to the untreated condition and demonstrates that the **neurons** present in the model are **mature and functional**.

# Results

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## A fully human innervated reconstituted epidermis

### Proteomic study

Significant differences in the relative abundance of 84 proteins :75 proteins up-regulated (ratio  $\geq 2$ ) and 9 proteins down-regulated (ratio  $\leq 0.5$ )

- Neurons seem still active
- Cells modifications, proliferation and migration could be activated.
- Neuronal development could be activated (neuromodulin, MAP1, neurofascin, DPYL2...)
- Gene expression and molecules transport could be activated, showing also a higher activity in the rebuilt epidermidis supplied with nerves

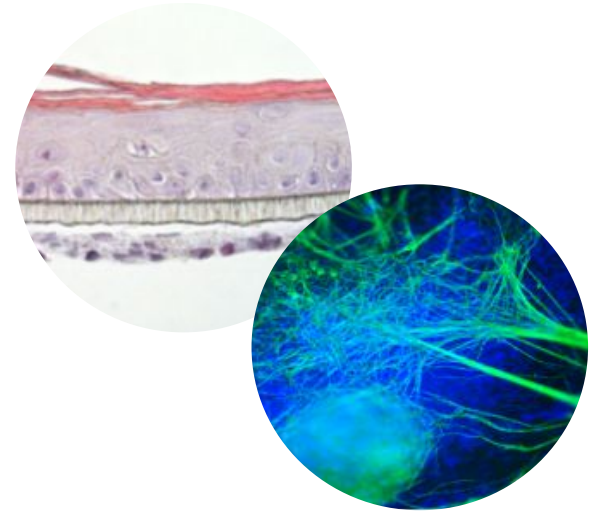
# Results

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## A fully human innervated reconstituted epidermis

### RHE-huSN

- Morphology and cellular organisation
  - histology
  - immuno-labelling
- Mature and functional neurons
  - CGRP release
  - neurons seem still and neuronal development could be activated (proteomics)



→ **relevant 3D model for evaluation of soothing effect of cosmetic actives**



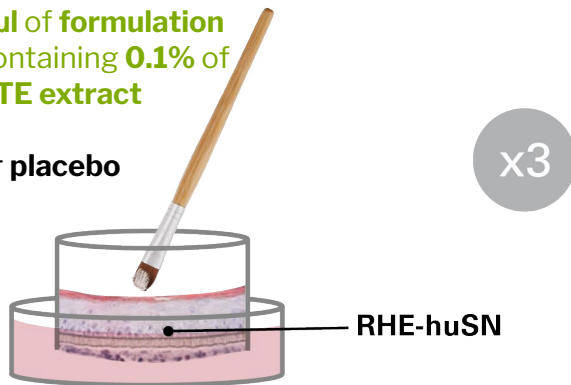
# An innovative 3D model

## *In vitro* study

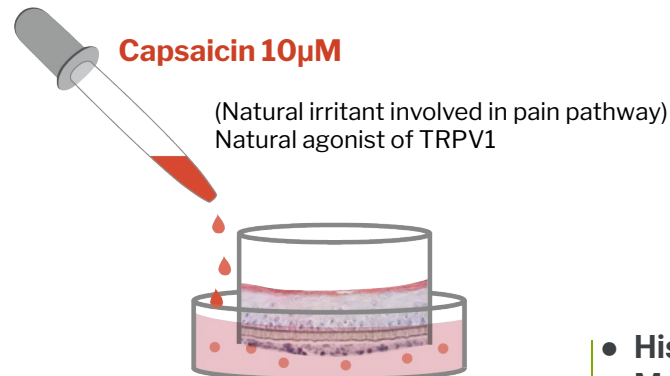
Pretreatment:

**2 $\mu$ l of formulation**  
containing **0.1% of**  
**ATE extract**

or **placebo**



t0



24h

20'

- Histology & immuno-labelling
- Measurement of neuropeptide (CGRP) release
- Proteomic analysis

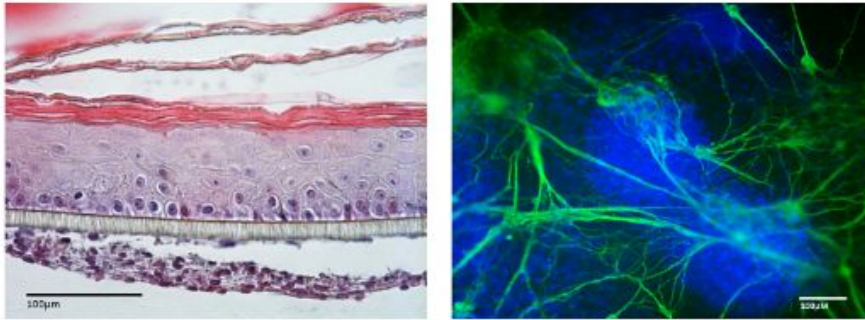
→ *Evaluation of soothing effect of cosmetic actives*



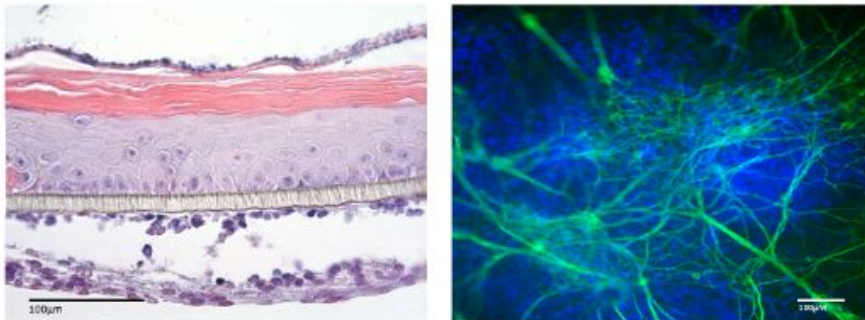
# Results

## Evaluation of ATE extract

Placebo  
+ Capsaicin 10 $\mu$ M



ATE extract 0.1%  
+ Capsaicin 10 $\mu$ M



### Histological analysis and immuno-labelling

➤ RHE-huSN sample, treated or not by ATE extract and/or capsaicin, has a morphology and cellular organization similar to RHE tissues grown in the absence of neurons

ATE extract (0.1%) **does not affect the viability and the morphology of the tissues.**

Histological analysis (hemalum/eosin staining) and immuno-labelling ( $\beta$ - tubulin: green and DAPI: blue) after capsaicin activation .

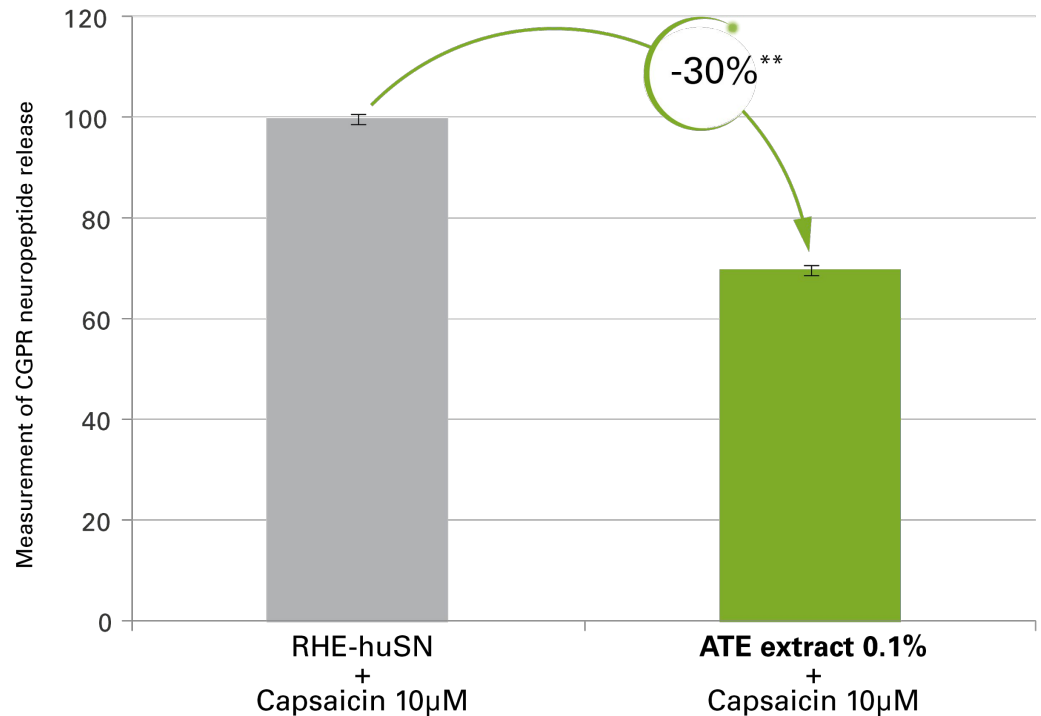
# Results

## Evaluation of ATE extract

### CGRP release

➤ ATE extract (0.1%) appears to be able to **downregulate the CGRP release** induced by capsaicin stimulation by 30%.

ATE extract (0.1%) soothing active



# Results

## Evaluation of ATE extract

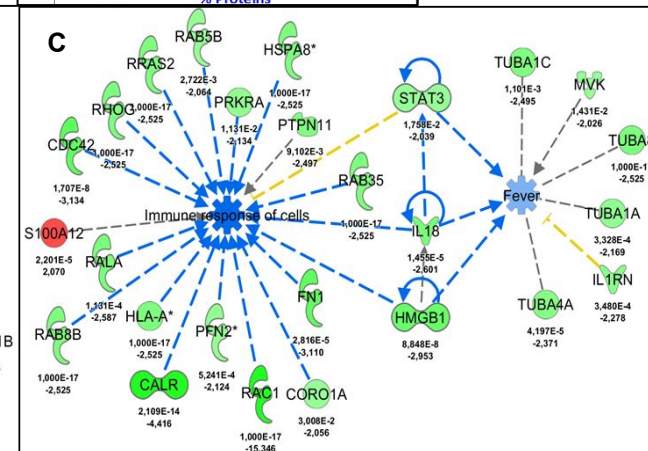
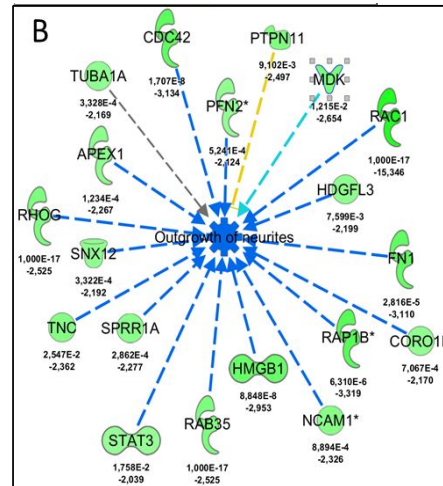
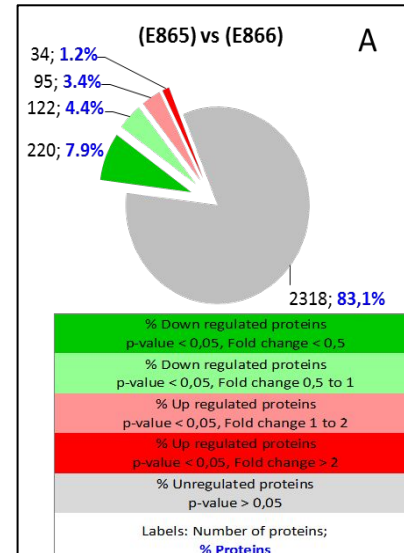
### Proteomic study

Significant differences in the relative abundance of 254 proteins: 220 proteins up-regulated (**ratio  $\geq 2$** ) and 34 proteins down-regulated (**ratio  $\leq 0.5$** ).

→ Neurites outgrowth could be limited (down-regulation of 19 proteins as TUB1A)

→ Inhibition of BDNF signaling pathway

→ Inflammatory response could be regulated (STAT3, IL-18, HMGB)



# Conclusion



## A fully human innervated reconstituted epidermis

- **Morphology and cellular organization** similar to RHE tissues grown in the absence of neurons
- **Mature and functional neurons**  
→ **increase of the neuropeptide CGRP release** under activation with capsaicin
- **Proteomic analysis have shown :**
  - active neurons and neuronal development
  - higher activity in RHE-huSN vs RHE

⇒ **Relevant for evaluation of soothing effect of cosmetic actives**

## Soothing properties of ATE extract

- Downregulation of CGRP release induced by capsaicin stimulation.
- Downregulation of neurites outgrowth which can lead to a decrease in the density of innervation which, linked with the inhibition of CGRP release, leads to a decrease in the skin sensitivity.
- Inhibition of BDNF signaling pathway that can lead to a decrease of neuron dendricity and then participate to a decrease of itching.
- Regulation of inflammation.

⇒ **Relevant for topic treatment and prevention of sensitive skin.**



# Thank you!



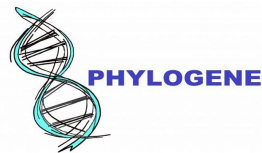
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