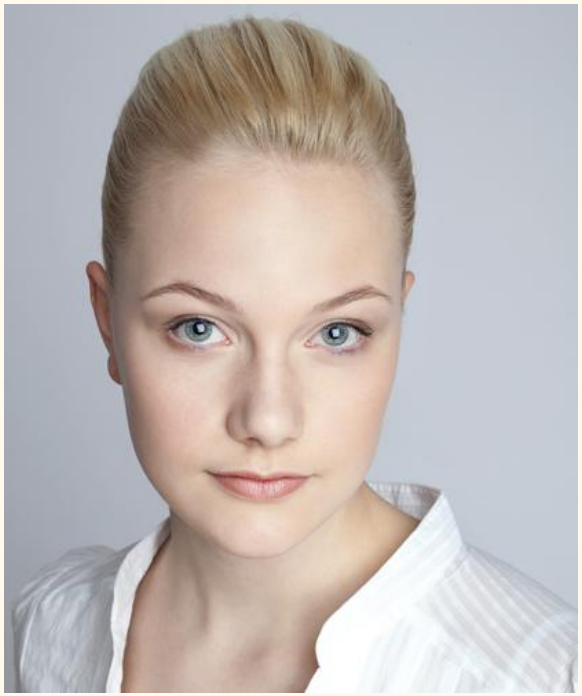
Scancos 2010



Evaluating the activity of a new plant-based soothing active

- Why design a new soothing active?
- Product Brief
  - Goals and ideas
- In Vitro
  - Initial screening
  - Primary testing – Inflammation in-vitro
  - Cellular resilience
  - Photosensitivity
  - Hyperosmotic shock
  - Barrier function ECIS
- In Vivo
  - Histamine
  - TEWL
  - Erythema
- Summary

Why design a new soothing active?

CLR gap filling

Market demands

Opportunity

Trend forecasting

New testing hardware

Niche areas of efficacy

Product Brief

Goals

Safe, significant measurable effects, natural, unique/protectable

Project Candidates

Phragmites kharka (Reed)

Poria Cocus *(* Mushroom*)*

Variables

Raw material

Source, growing conditions, part of plant, sub-species

Manufacture

Isolation, separation, physical form, carrier

Synergism

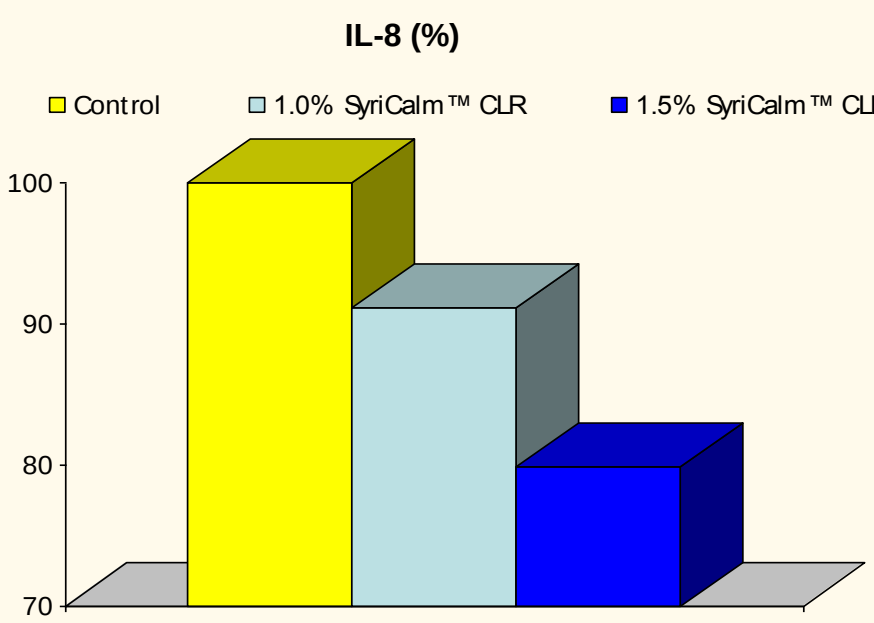
Targeted combinations

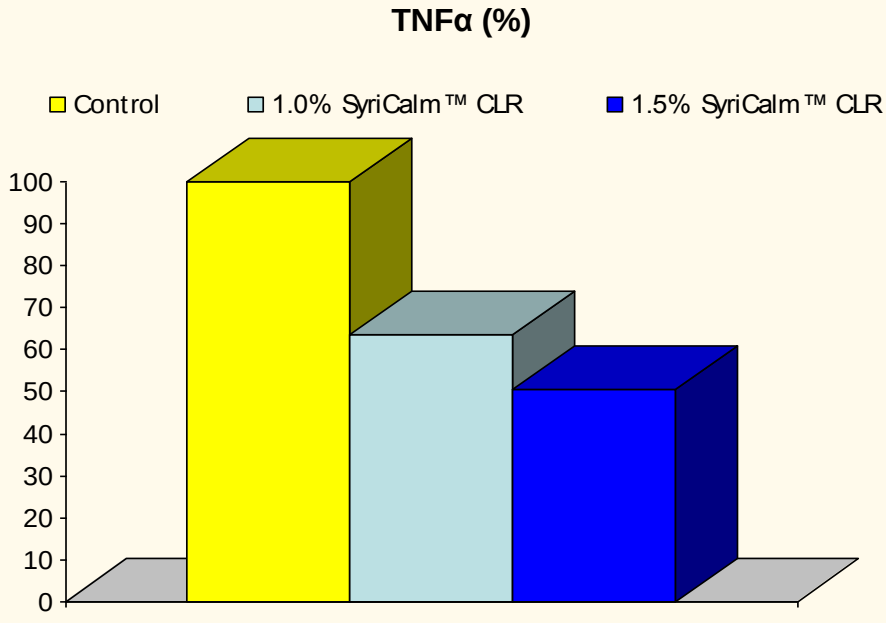
SyriCalm™ CLR

In vitro Test Results

Effect on UV-induced inflammation

Reduction of UV-induced proinflammatory mediator production





Keratinocytes were irradiated with UV light (2 J/cm² UVA; 0.2 J/cm² UVB) after pretreatment with different dosages of SyriCalm™ CLR.
The release of IL-8 and TNFα by irradiated cells without pretreatment with SyriCalm™ CLR is set at 100%.
Method: Luminescence ELISA

SyriCalm™ CLR

In vitro Test Results

Cellular resilience against

UV irradiation

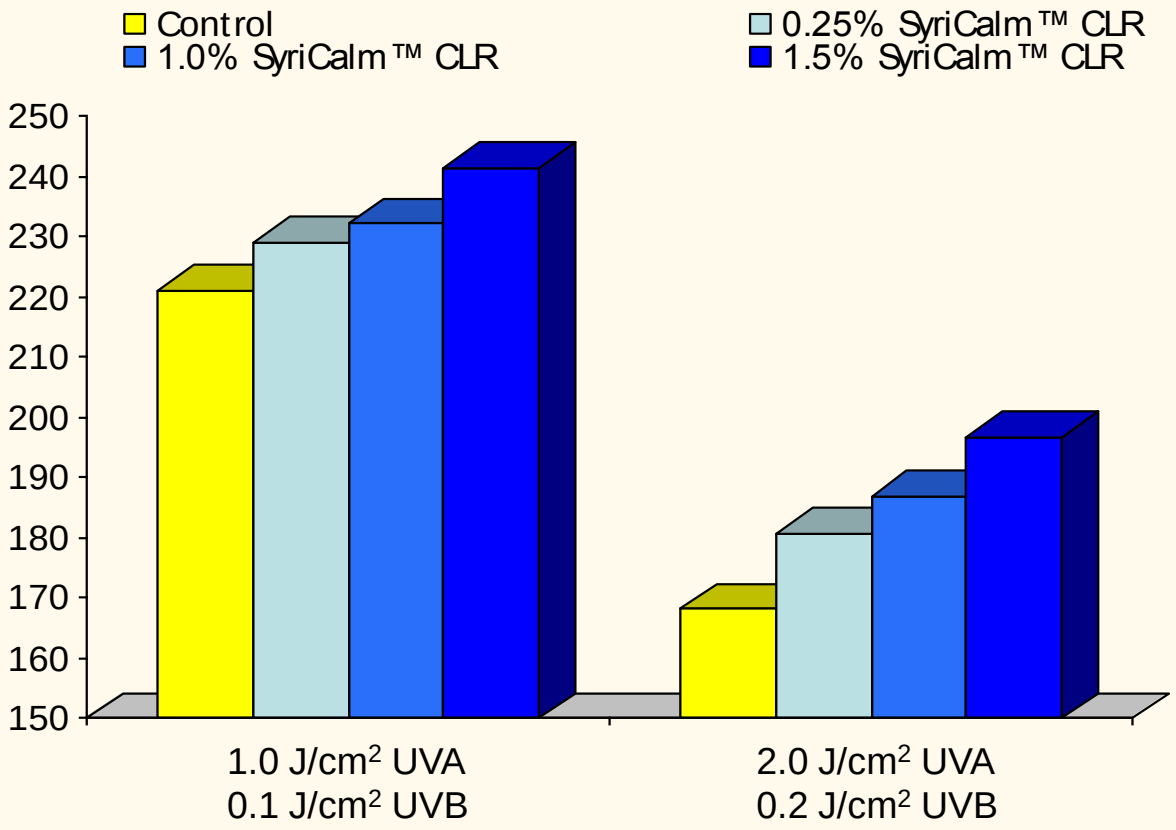
Protection against UV-induced ATP depletion

ATP (RLU)

Keratinocytes were irradiated with different dosages of UV light after pretreatment with different dosages of SyriCalm™ CLR.

Control cells were irradiated without pretreatment with SyriCalm™ CLR.

Method: Luciferase/Luceferin assay

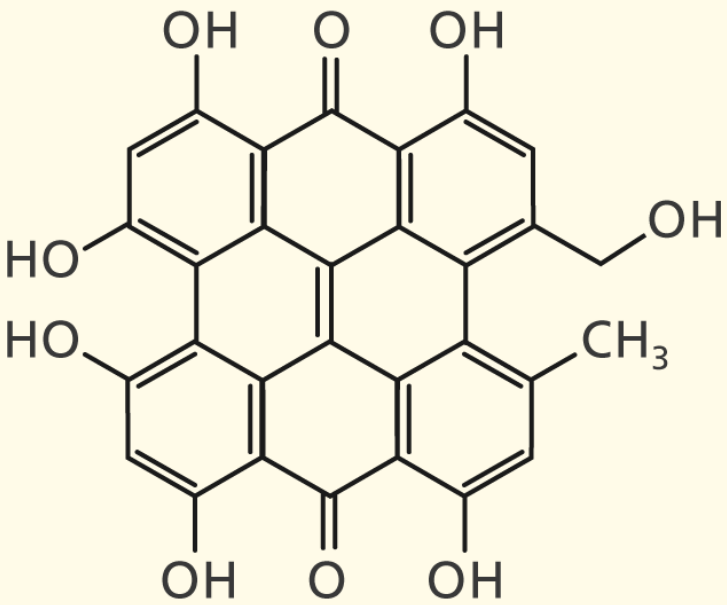


In vitro Test Results

Effect on Photosensitivity

Photosensitivity

Hypericin



UV

Reactive oxygen species

- Decrease in cell viability
- Inflammation

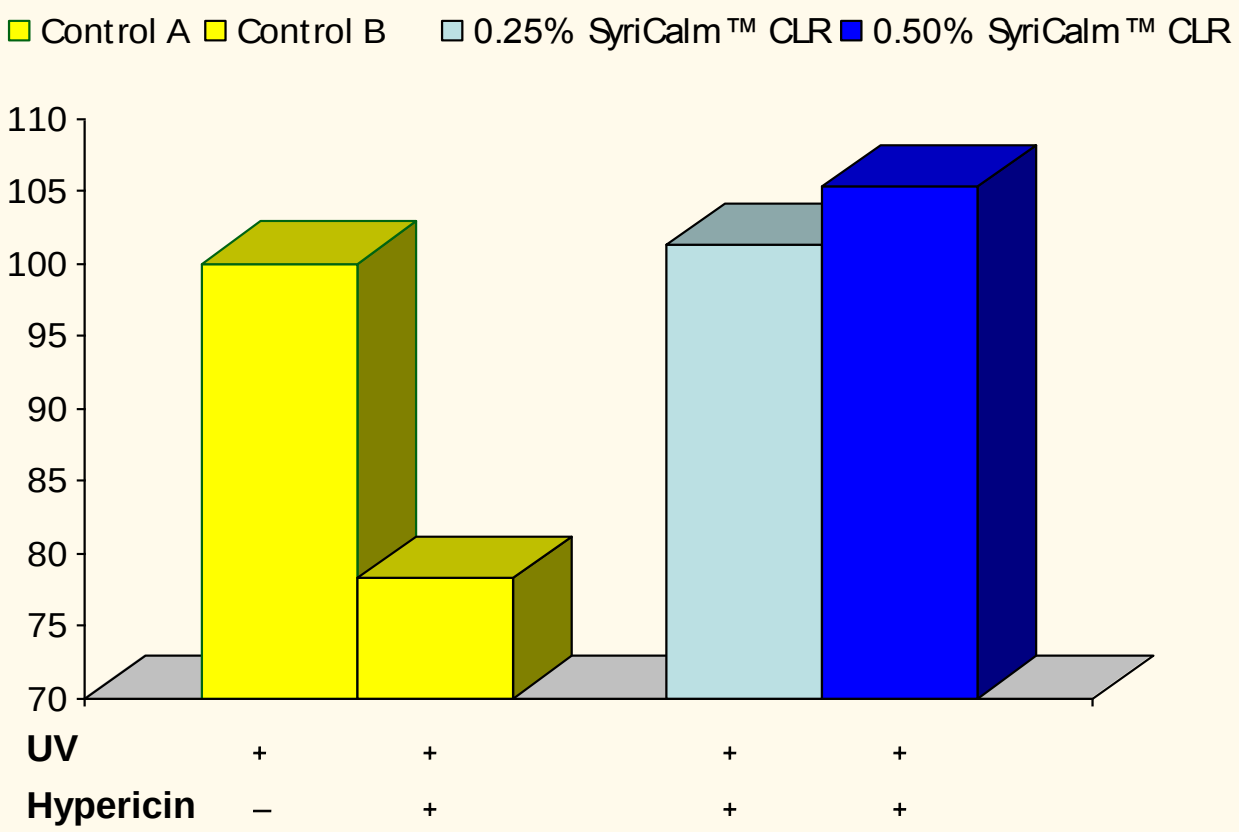
Hypericin phototoxicity: Protection against decrease in cell viability

Cell viability (%)

Keratinocytes were treated with hypericin (0.5 µM) and UV irradiation (0.25 J/cm² UVA; 0.025 J/cm² UVB) with and without pretreatment with SyriCalm™ CLR.

Related to control, without presence of hypericin and SyriCalm™ CLR (100%).

Method: MTT assay (absorption: 570 nm)



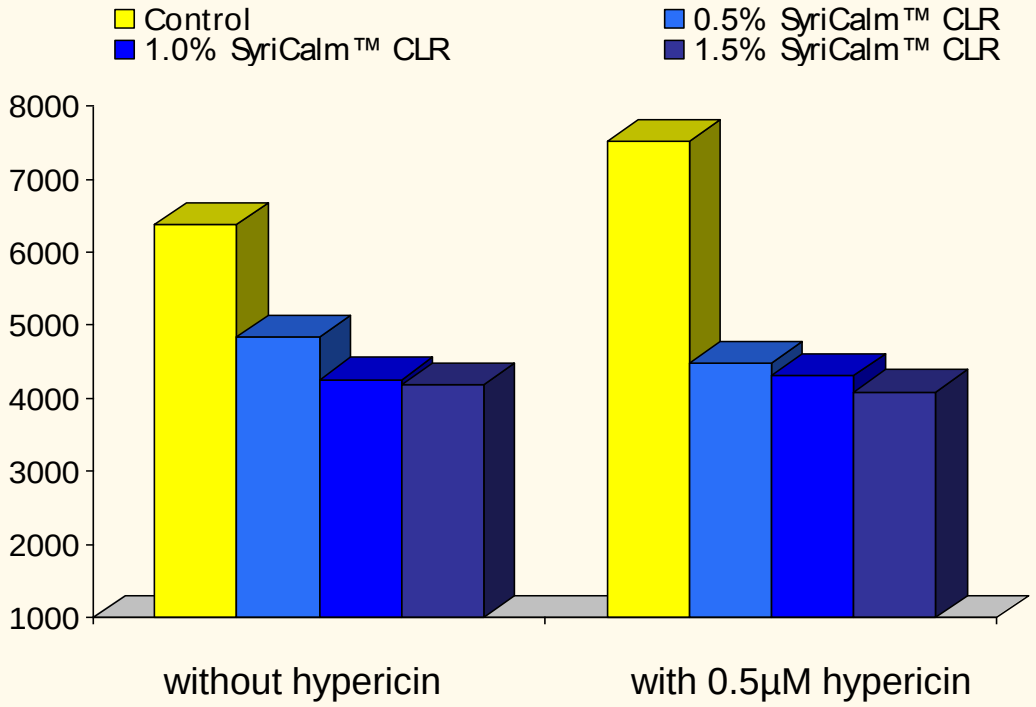
Hypericin phototoxicity: Reduction of IL-8 expression

IL-8 (RLU)

Keratinocytes were treated with and without SyriCalm™ CLR, then irradiated with 0.25 J/cm² UVA; 0.025 J/cm² UVB.

RLU (relative luminescence units)

Method: Luminescence ELISA

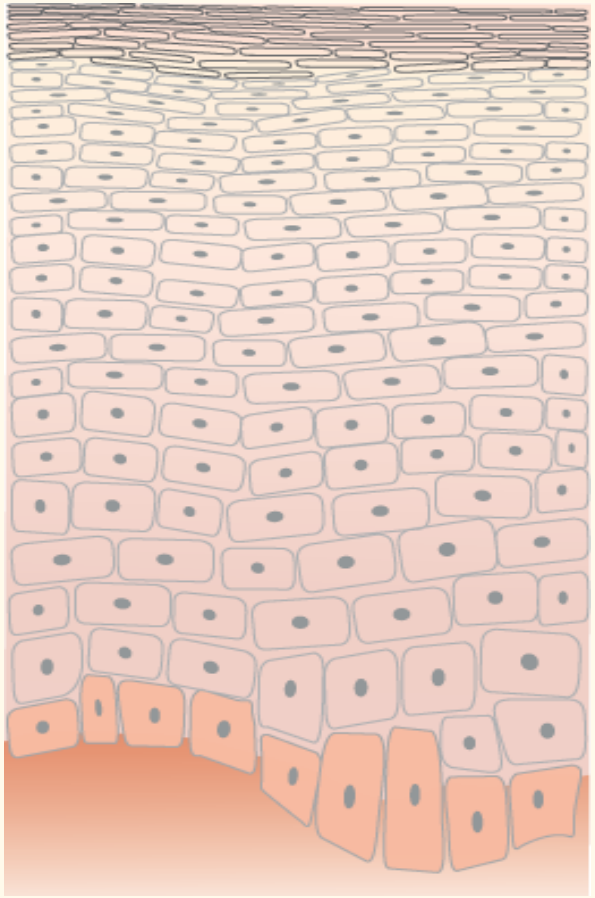


Epidermal integrity

The barrier function of the skin is not only dependent on the quality of the Stratum Corneum.

External stress leads to alterations in cell shape and volume and intercellular anchoring in the epidermis

- loss in barrier function
- inflammation



SyriCalm™ CLR

In vitro Test Results

Effect on cellular Taurine efflux during hyperosmotic stress

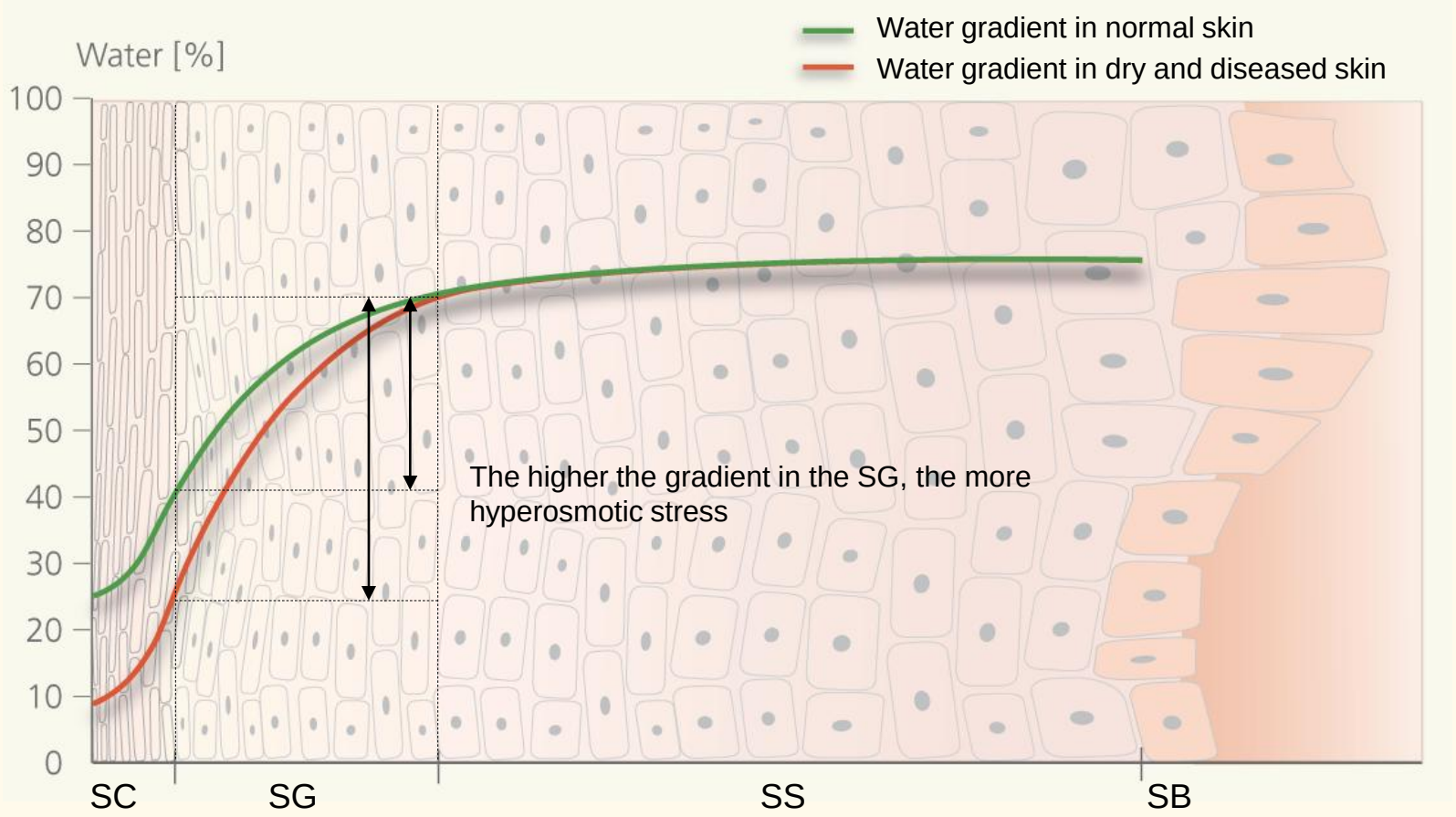
Hyperosmotic stress - consequences

Keratinocytes in the Stratum Granulosum are subjected to constantly fluctuating osmotic conditions →

- degradation of intra- and intercellular protein structures
- impaired protein synthesis
- impaired DNA repair mechanisms
- induction of apoptotic mechanisms
- further loss of barrier function, etc.

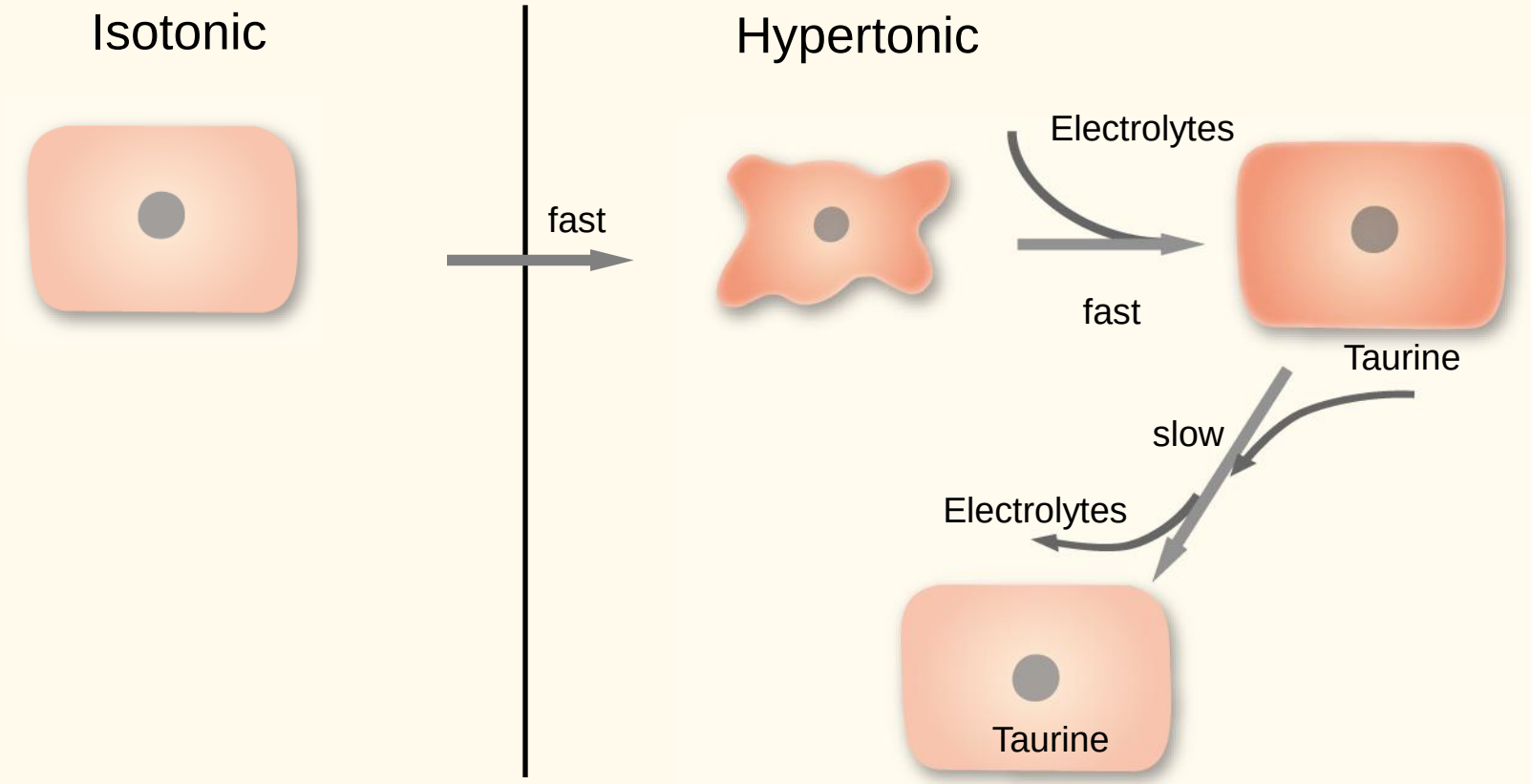
Healthy keratinacytes in the SG are vital to skin function.

Hyperosmotic stress in the epidermis

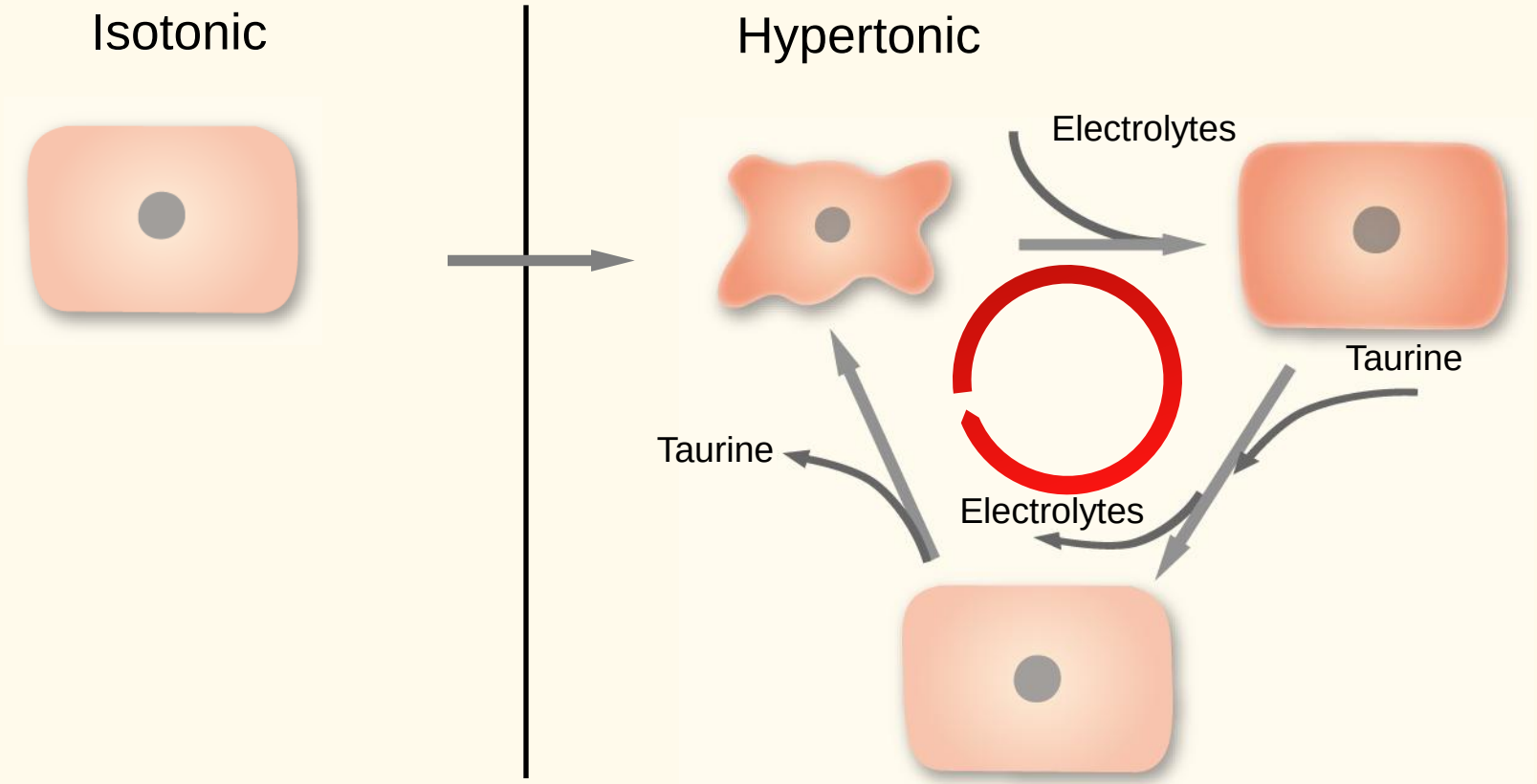


SyriCalm™ CLR

Hyperosmotic stress - Strategies

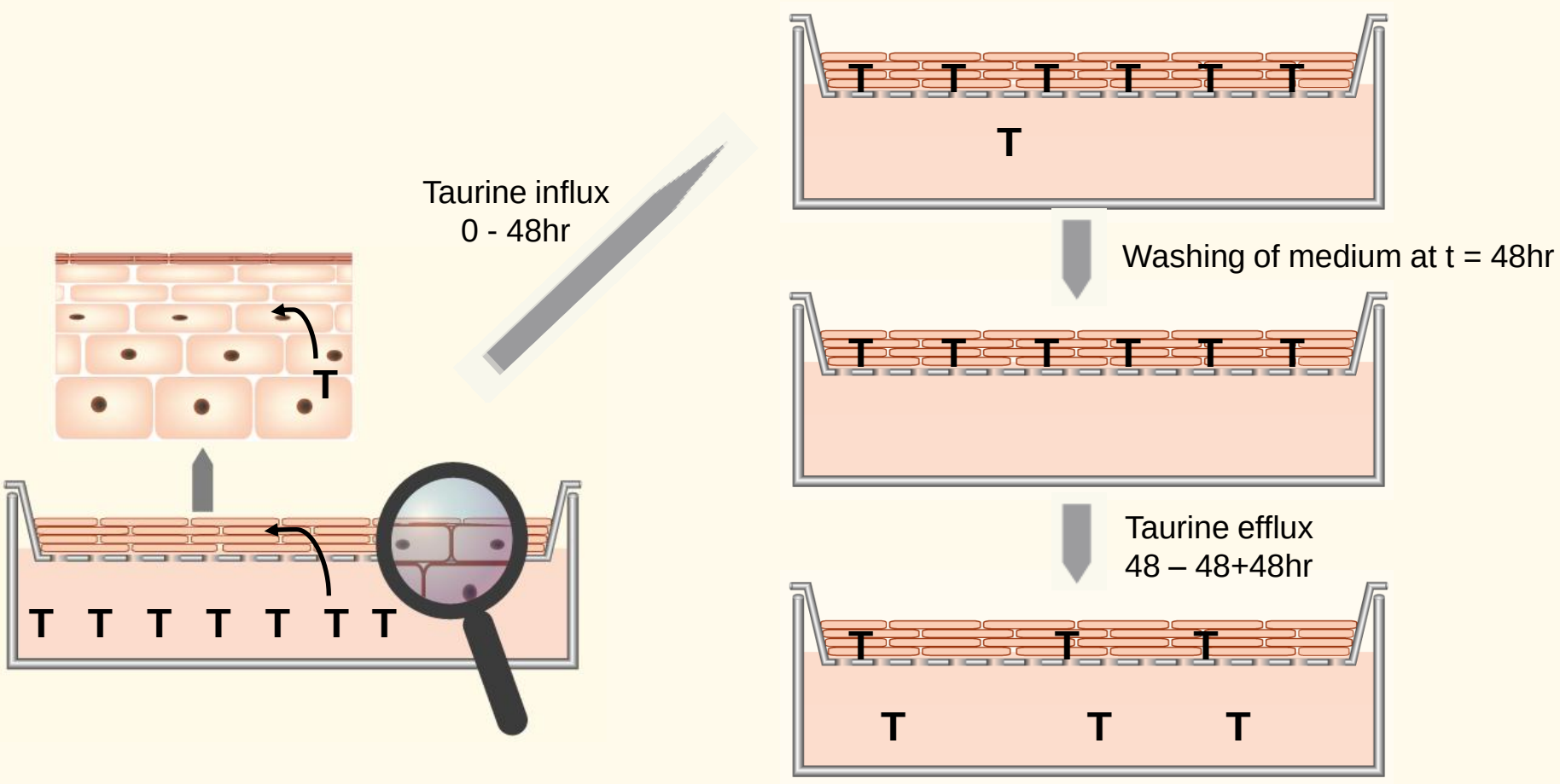


Hyperosmotic stress - Challenges



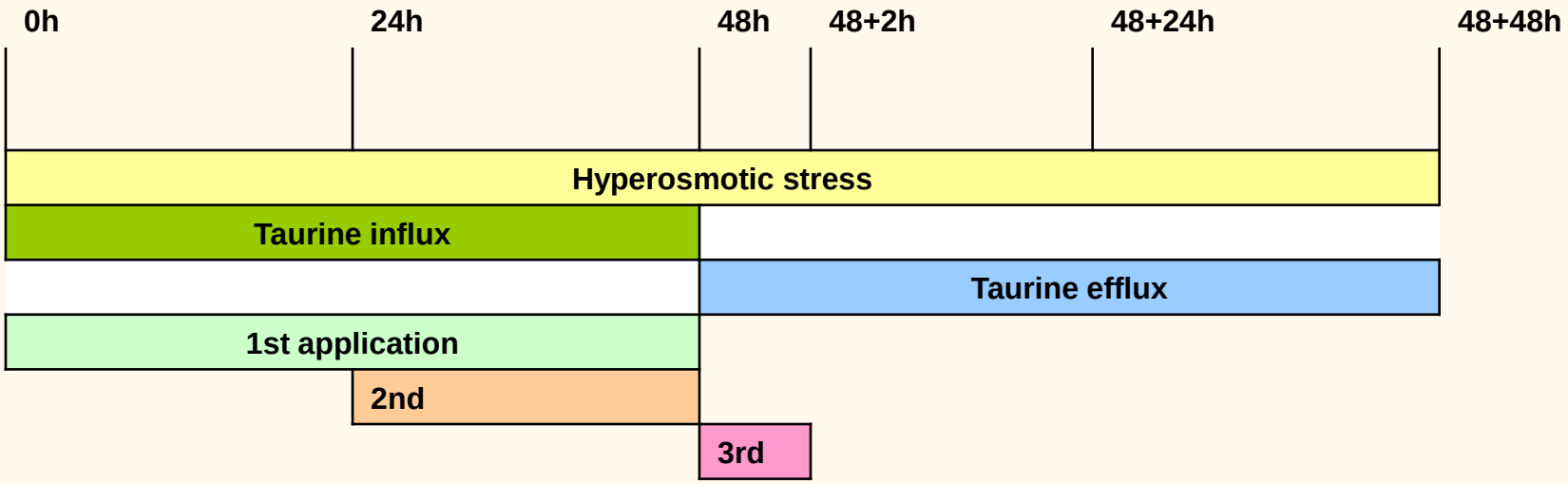
SyriCalm™ CLR

Experiment – Protection against Taurine efflux



SyriCalm™ CLR

Experiment – Protection against Taurine efflux



Method: evaluation of counts per minute with TopCount NXT™ Microplate Scintillation counter (Perkin elmer) in MicroScint 40 Scintilation cocktail (Perkin Elmer)

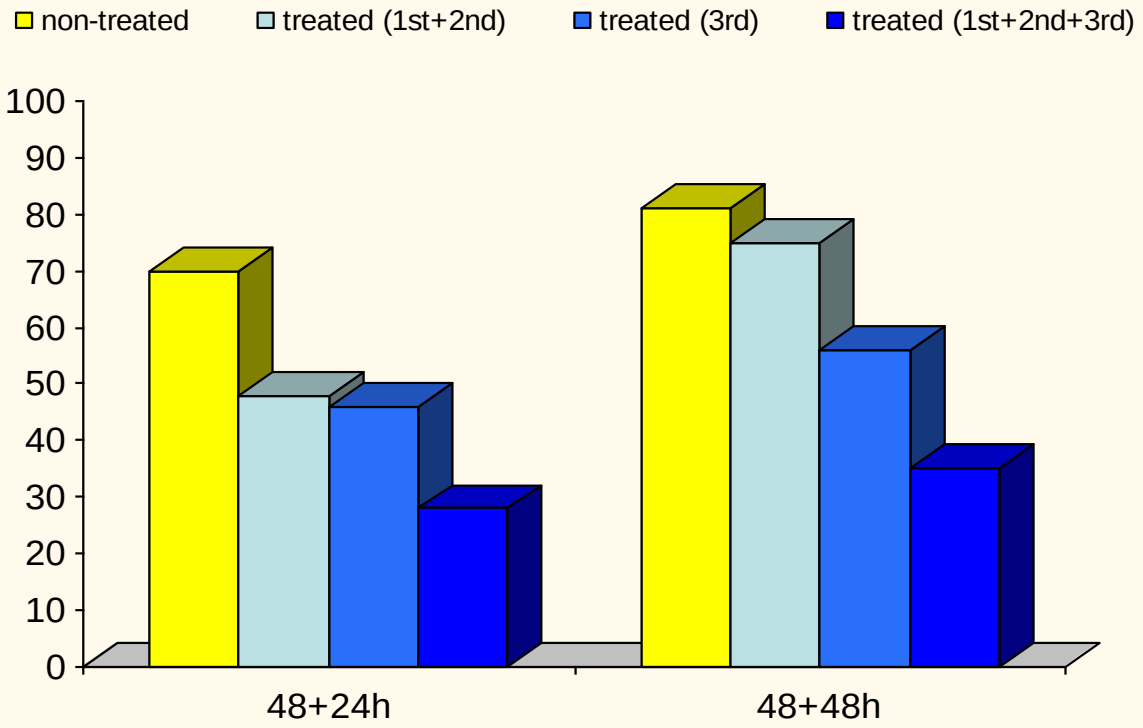
Protection against Taurine efflux

Taurine efflux (%)

3D epidermal skin models were established in a CELLnTEC PCT medium, after which they were exposed to a hyperosmotic medium (400 mOsm) and treated with SyriCalm™ CLR and analyzed according mentioned scheme.

The osmolarity of the medium was kept at 400 mOsm during the whole experiment.

Taurine efflux is expressed as percentage radioactivity of the cell medium related to total radioactivity (cell lysate + consecutive samples of the medium)

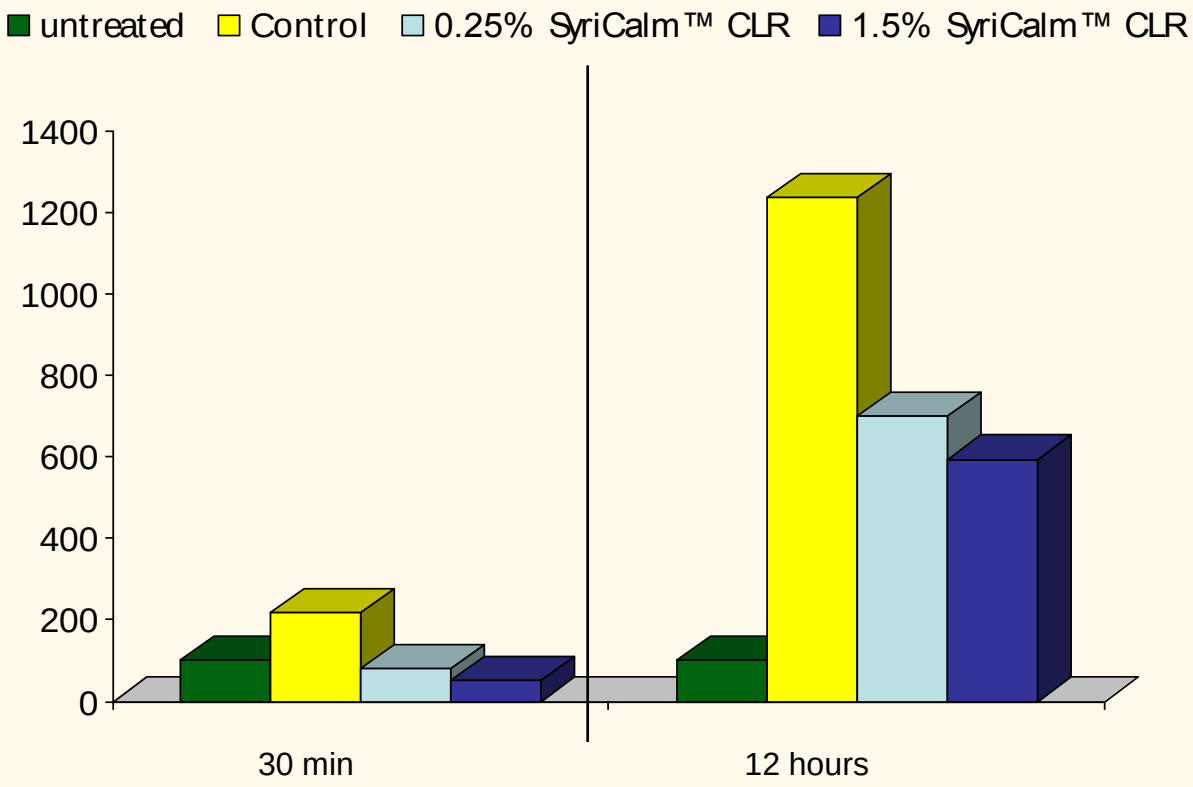


Protection against IL-8 expression during hypo-osmotic stress

IL-8 (%)

Keratinocytes were incubated in a hypo-osmolary medium (250 mOsm) for 30 min and 12 hours in the presence of different concentrations of SyriCalm™ CLR.

Method: Luminescence ELISA



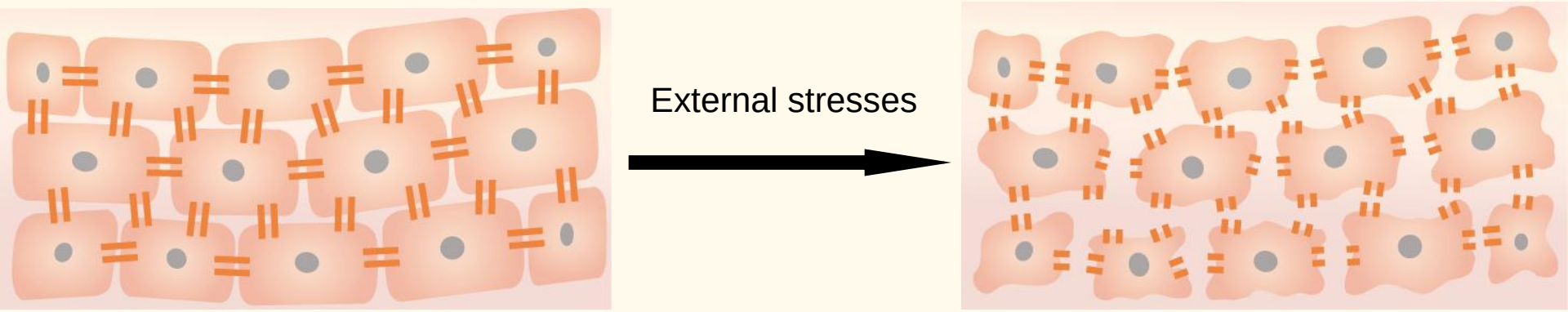
SyriCalm™ CLR

In vitro Test Results

Effect on epidermal integrity

Determining epidermal integrity with ECIS

External stress leads to a disturbance of epidermal integrity
- loss in cellular volume
- degradation of intra- and inter cellular structural proteins, potentially leading to cell-detachment



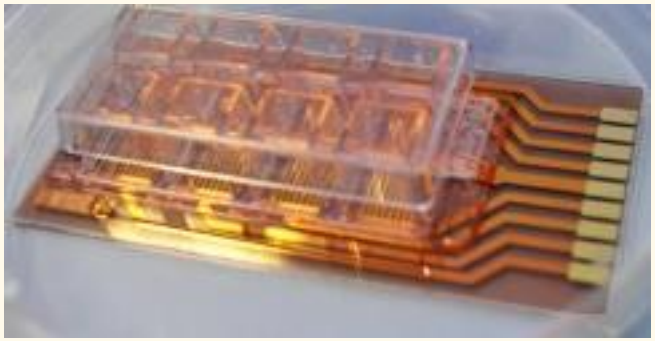
Determining epidermal integrity with ECIS

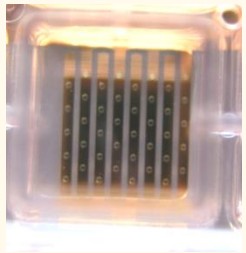
ECIS: Electric Cell-substrate Impedance Sensing

An automated non-invasive method to monitor cell behaviour and attachment (barrier function)

Principle:

A confluent layer of keratinocytes will have a certain resistance (impedance) against conducting an electrical current.
Change in cell volume and shape and loss of intercellular attachment lead to a loss of impedance. Regaining the impedance is a measure for regaining barrier function.

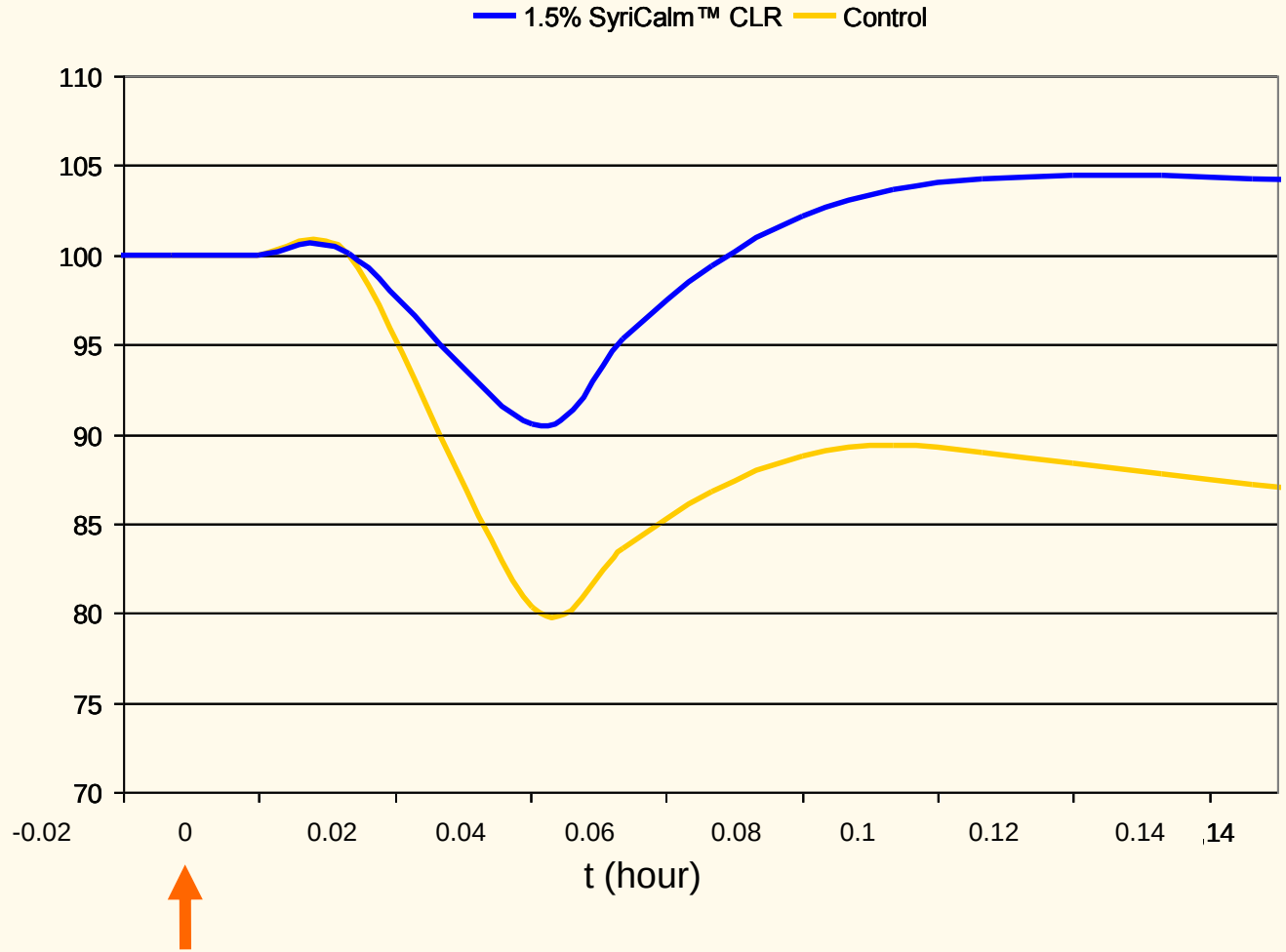




Protection against loss of epidermal integrity after an electrical pulse

Change in Impedance (%):

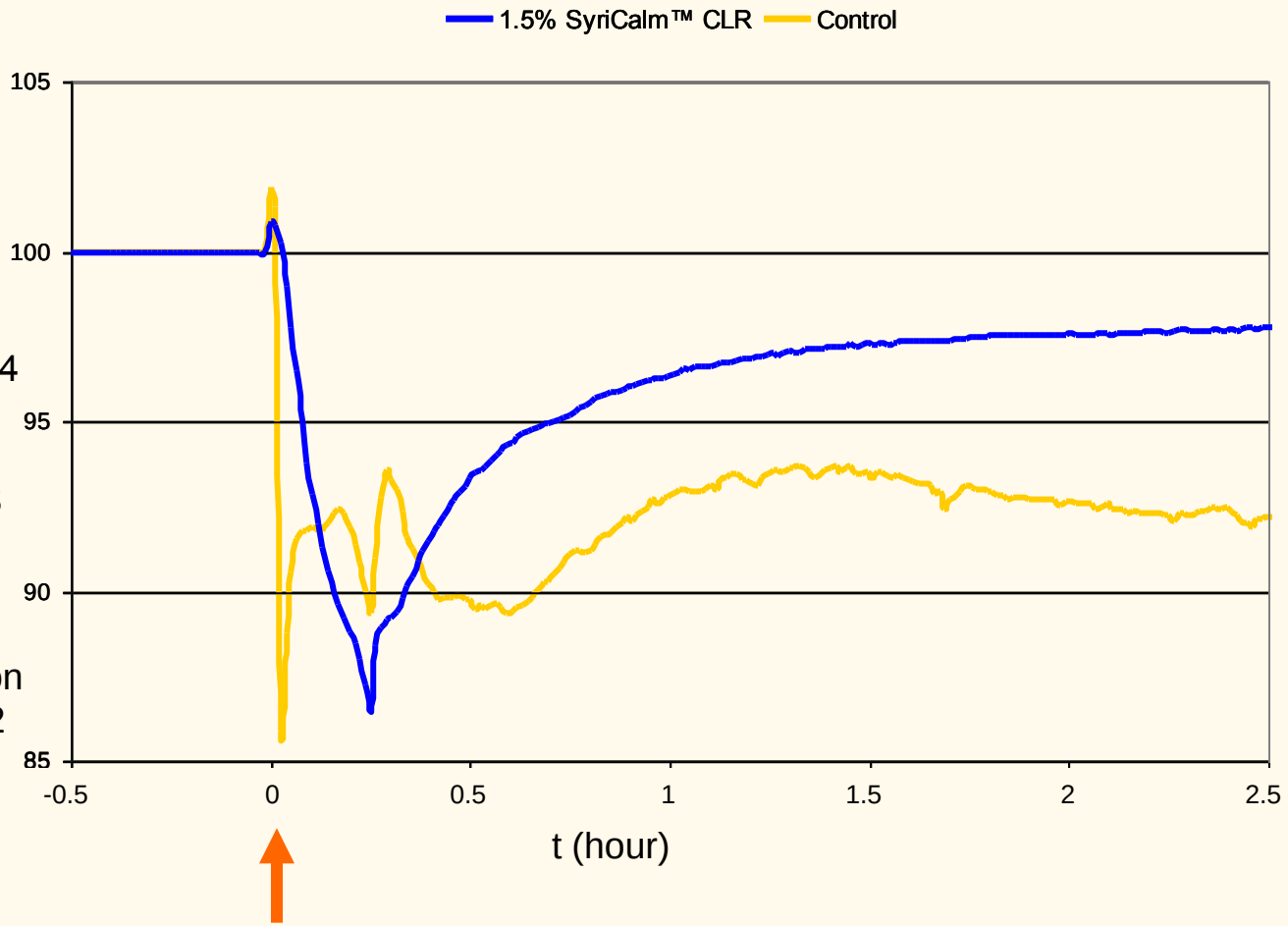
A confluent layer of Human Keratinocytes was applied on an Electrode Array Chip and incubated with SyriCalm™ CLR for 24 hours without FCS. The chip was connected to an ECIS instrument. Values of resistance were collected before and after an Elevated Field Pulse was applied (t=0, 15 kHz, 6 V, 5 sec). The impedance before the pulse is set at 100%.



Protection against loss of epidermal integrity after UV irradiation

Impedance (%)

A confluent layer of Human Keratinocytes was applied on an Electrode Array Chip and incubated with SyriCalm™ CLR for 24 hours without FCS. The chip was connected to an ECIS instrument. Values of resistance were collected before and after UV irradiation (t=0, 2 J/cm² UVA+0.2 J/cm² UVB). The impedance before the irradiation was set at 100%.



SyriCalm™ CLR

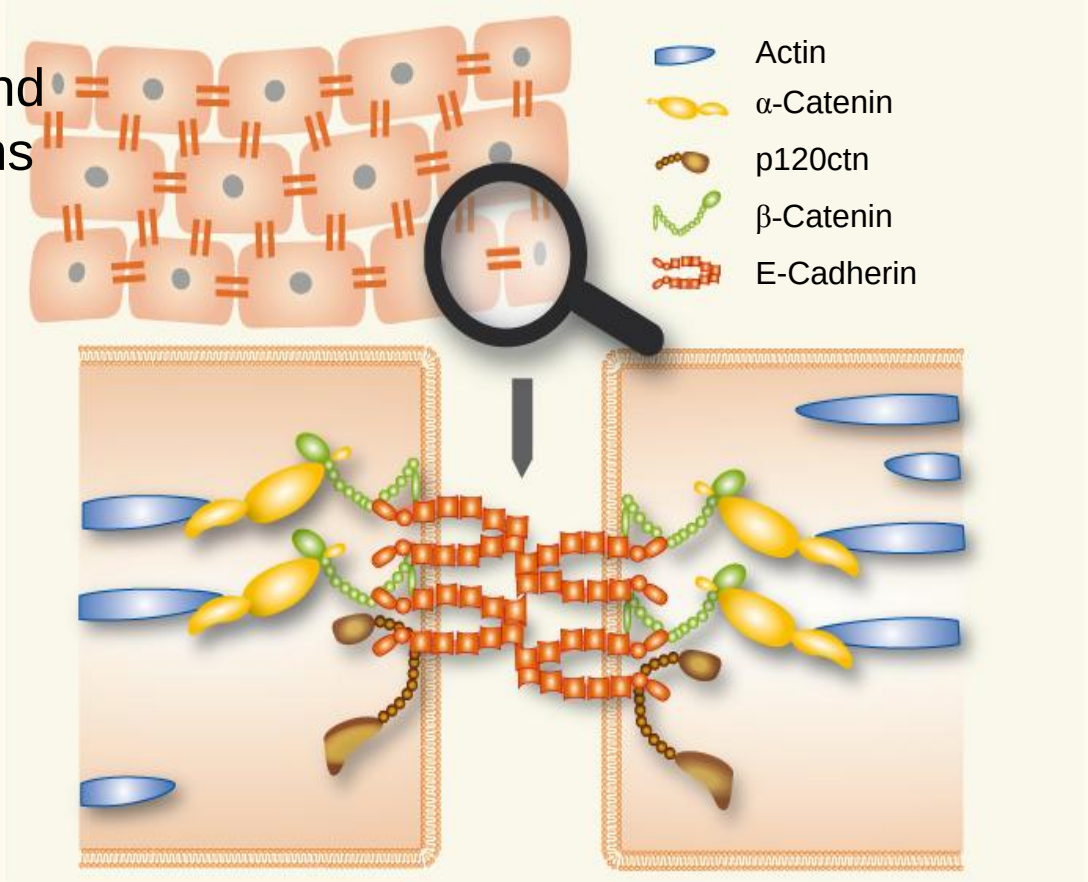
In vitro Test Results

Effect on UV induced degradation of E-Cadherin

E-Cadherin in the epidermis

Trans-membrane protein, essential in the formation and maintenance of cell junctions in the epidermis

- plays a crucial role in the formation of tight junctions and desmosomes
- part of adherens junctions

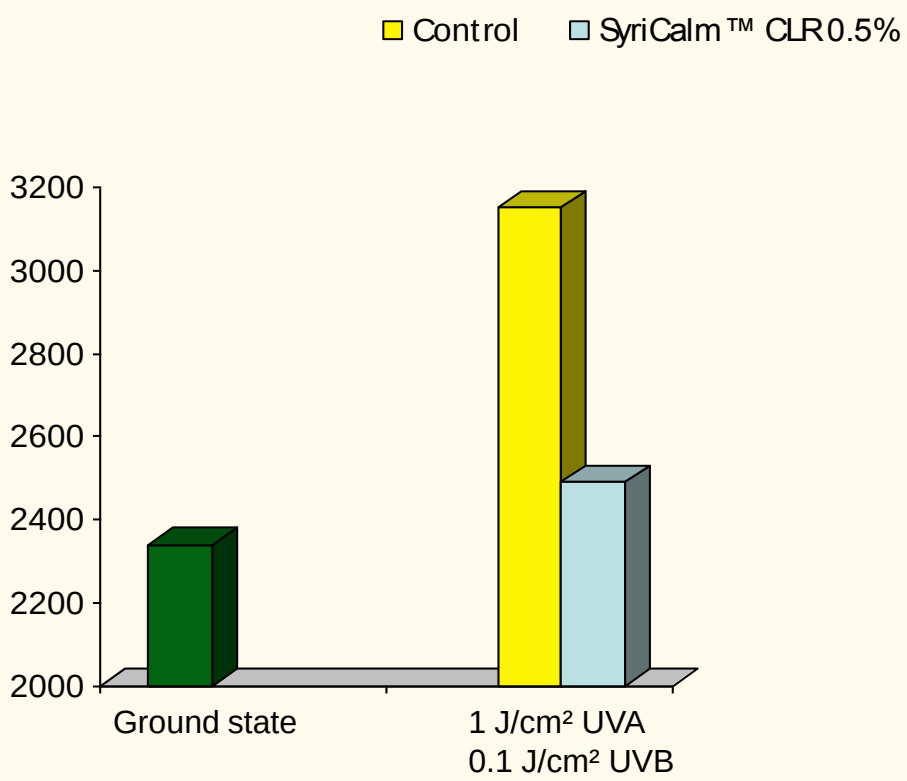


Reduction of UV-induced E-Cadherin degradation

Soluble E-Cadherin (pg/ml)

Keratinocytes were irradiated with UV light (1 J/cm² UVA; 0.1 J/cm² UVB) after pretreatment with 0.5% of SyriCalm™ CLR.

Method: ELISA



SyriCalm™ CLR

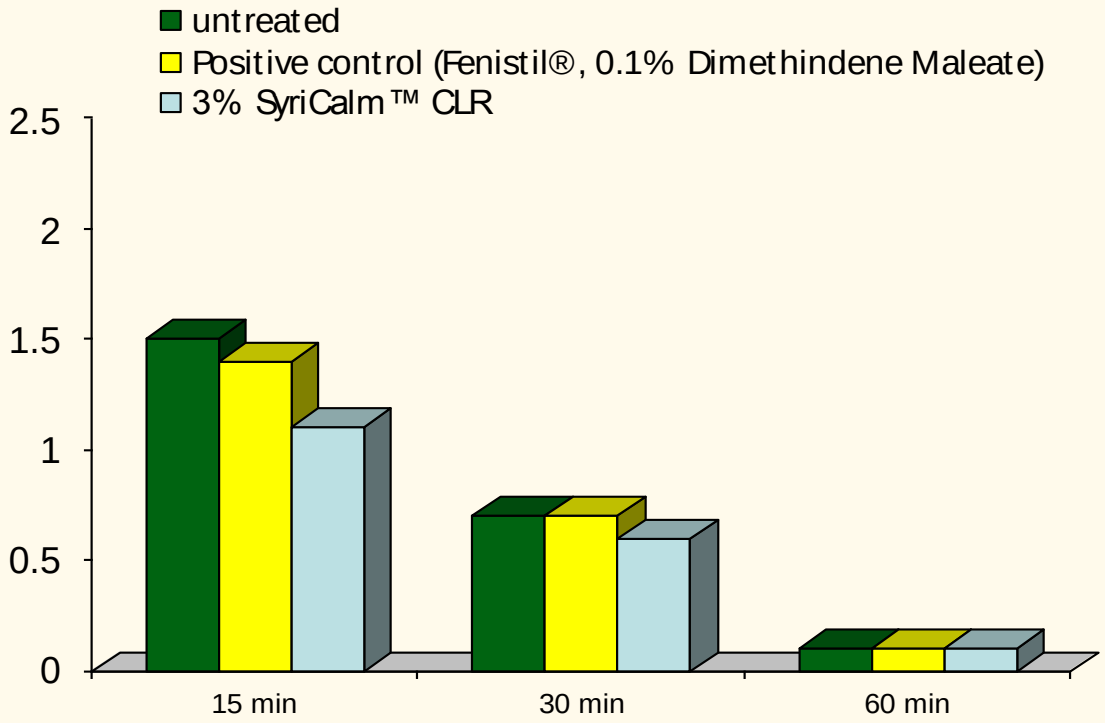
In vivo Test Results

Effect on histamine-induced itching

Effect on histamine-induced itching

Itching (Scoring system)

Histamine was applied intracutaneously on a designated area on the inner forearm of 10 volunteers (33–79 years old) via a lancet stitch. The application of the test product took place immediately after the insult with histamine. The itching was assessed by a scoring system
15, 30 and 60 min after application of the test product.



SyriCalm™ CLR

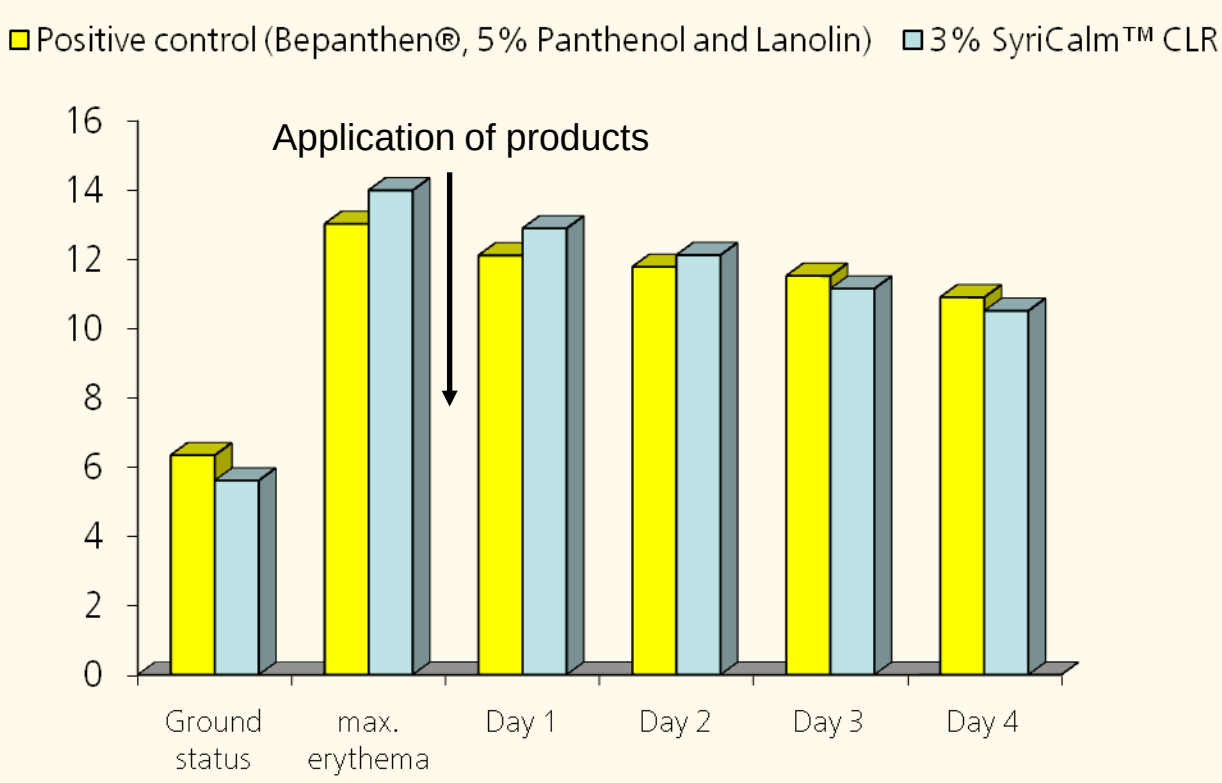
In vivo Test Results

Effect on UV-induced increase
in TEWL

Effect on UV-induced increase in TEWL

TEWL (g/hm²)

On designated skin areas on the inner side of the thigh of 10 volunteers (33 - 79 years old) the TEWL was measured. Then these areas were exposed to
2.0 MED of UVB irradiation, inducing loss of barrier function. 24 hours after that the TEWL was measured again. Directly after that the test products were applied for 4 days, twice daily. Every day the TEWL was measured (method: Tewameter TM 210, Courage & Khazaka, Germany)



SyriCalm™ CLR

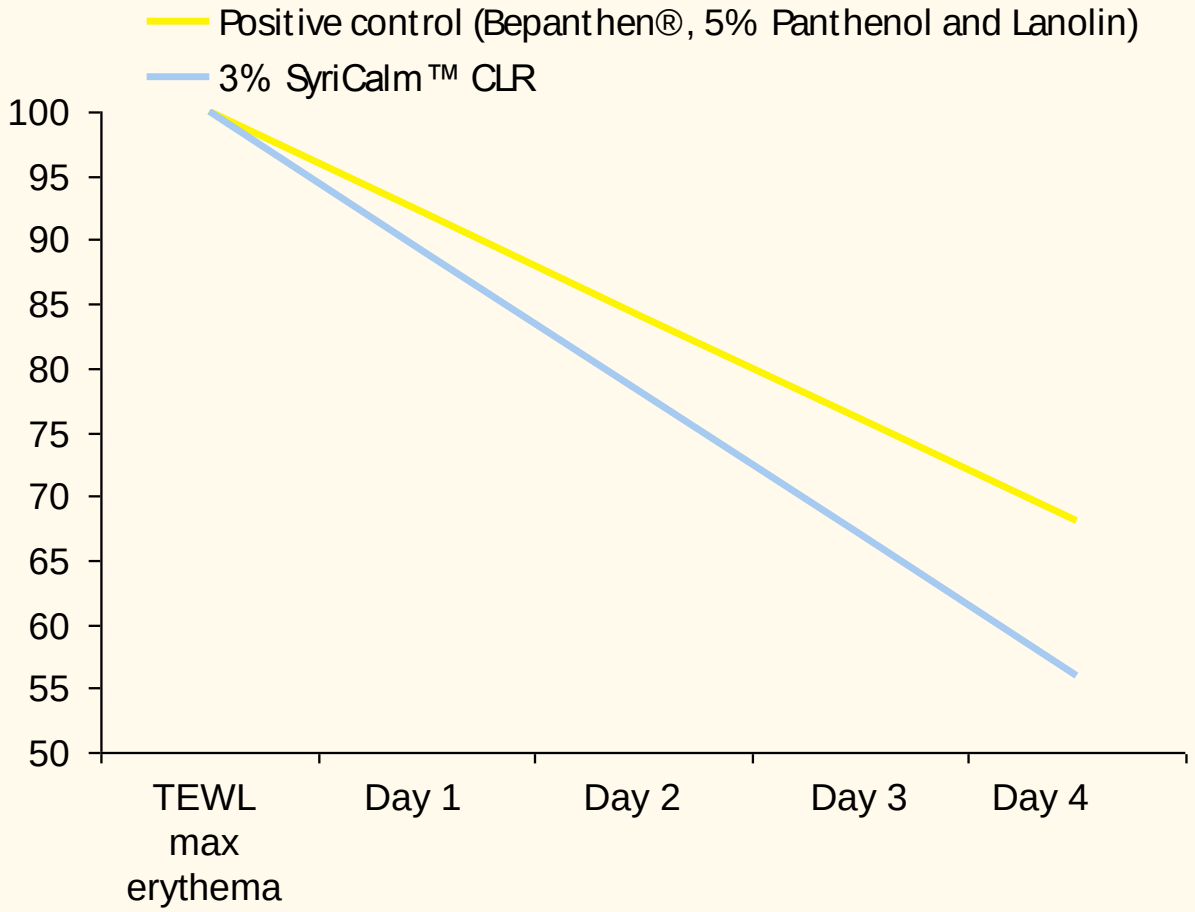
Effect on UV-induced increase in TEWL - Trends

TEWL (%)

Trends in reduction of TEWL as a function of time.

Comparison between 3% SyriCalm™ CLR and Positive control.

The TEWL measured at maximum Erythema was set at 100%.



SyriCalm™ CLR

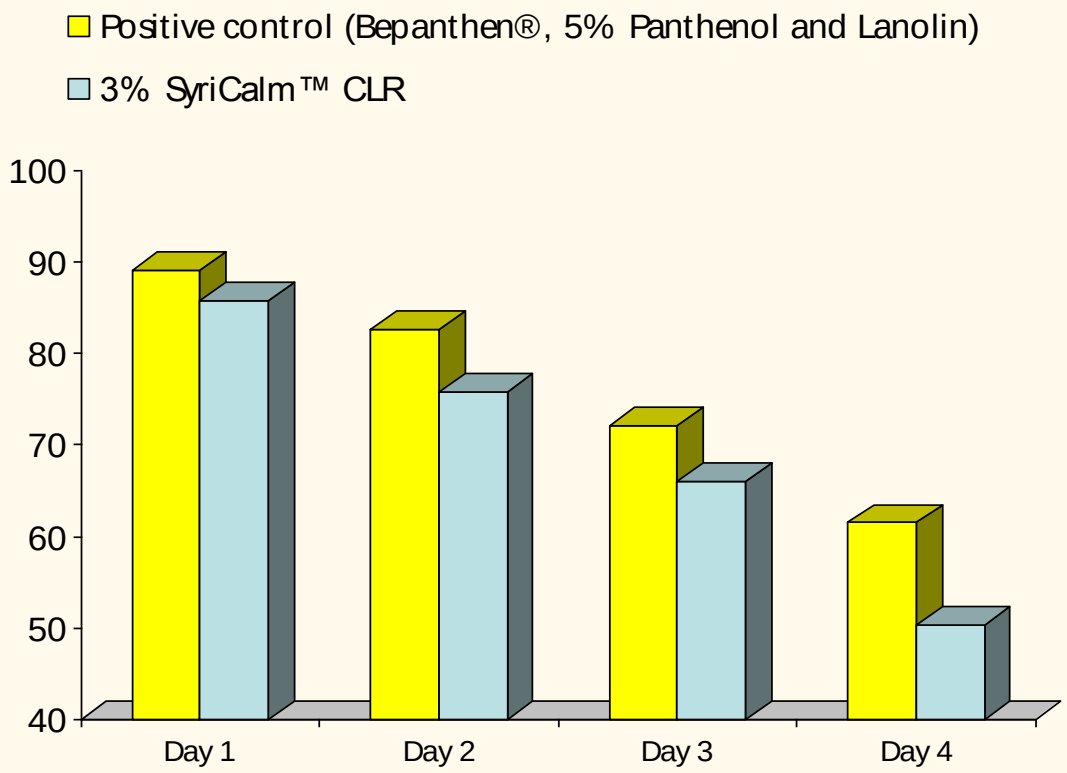
In vivo Test Results

Effect on UV-induced erythema

Effect on UV-induced erythema

Skin redness (%)

On designated skin areas on the inner side of the thigh of 10 volunteers (33 - 79 years old) the skin redness was measured. Then these areas were exposed to 2.0 MED of UVB irradiation, inducing erythema. 24 hours after that the redness of the skin was measured again. Directly after that the test products were applied for 4 days, twice daily. Every day the skin redness was measured. The maximum erythema is set at 100%. (Method: Chromameter CR 200, Minolta, Japan)



Summary

In vitro:

- Protection against external stresses
    - Increase in cell viability
    - Increase of cell energy level
    - Improvement of cellular structure
    - Supports barrier function
    - Fights against the effects of hyperosmotic stress
- Reduction of photosensitivity
- Anti-inflammatory action

In vivo:

- Protection against histamine & UV-induced stress
    - Accelerated recovery of skin barrier function (TEWL)
    - Accelerated reduction of erythema
    - Accelerated reduction in itching

INCI: Water, Phragmites Kharka Extract, Poria Cocos Extract

Dosage: 3%

Recommended pH: 3.0 – 8.0

Preserved with sodium benzoate