CLR Berlin and probiotic lysates, i.e. postbiotics

A true heritage

Company Background

Specialized in the development of high-quality, innovative cosmetic concepts for skin and hair care.

In-house R&D, formulation and manufacturing.

CLR · for fine cosmetics

- independent and privately owned
- 1926 founded in Berlin
- since the early 1950s focus on active cosmetic ingredients

CLR’s probiotic lysates: proven to be successful

At least 20 skincare products containing CLR‘s probiotic lysates are sold every minute.

Postbiotics at CLR

In the 50´s
Professor Martin Kludas from the Free University of Berlin starts research into beneficial effects of probiotic bacteria on the human body

In the 70´s
Kludas starts to look at the positive effect of probiotic bacteria on skin and gets in touch with Günther Borchert the founding father of **CLR** as it is now

In the late 70´s
Kludas and Borchert file for patents concerning the use of a lysate of probiotic bacteria for the benefit of anti-aging cosmetics, specifically including DNA repair

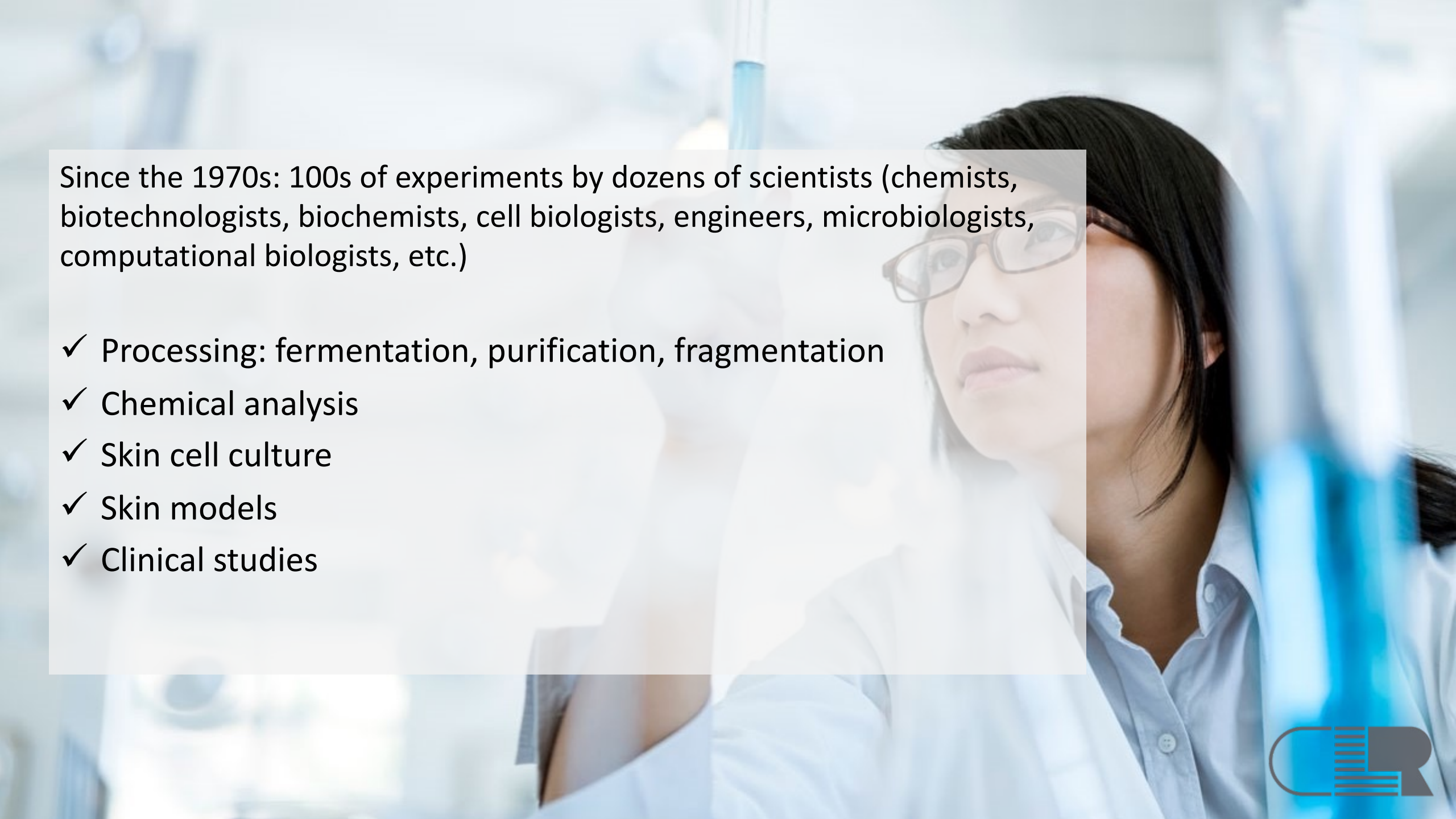
Early 80´s
CLR's probiotic lysates find their way in flagship brands of large cosmetic multinationals

1982
First version of Repair Complex CLR™ , research continues

2004
Introduction of ProBioBalance CLR™

2015
Introduction of ProRenew Complex CLR™, research continues further

2023
New G+C Complex CLR™

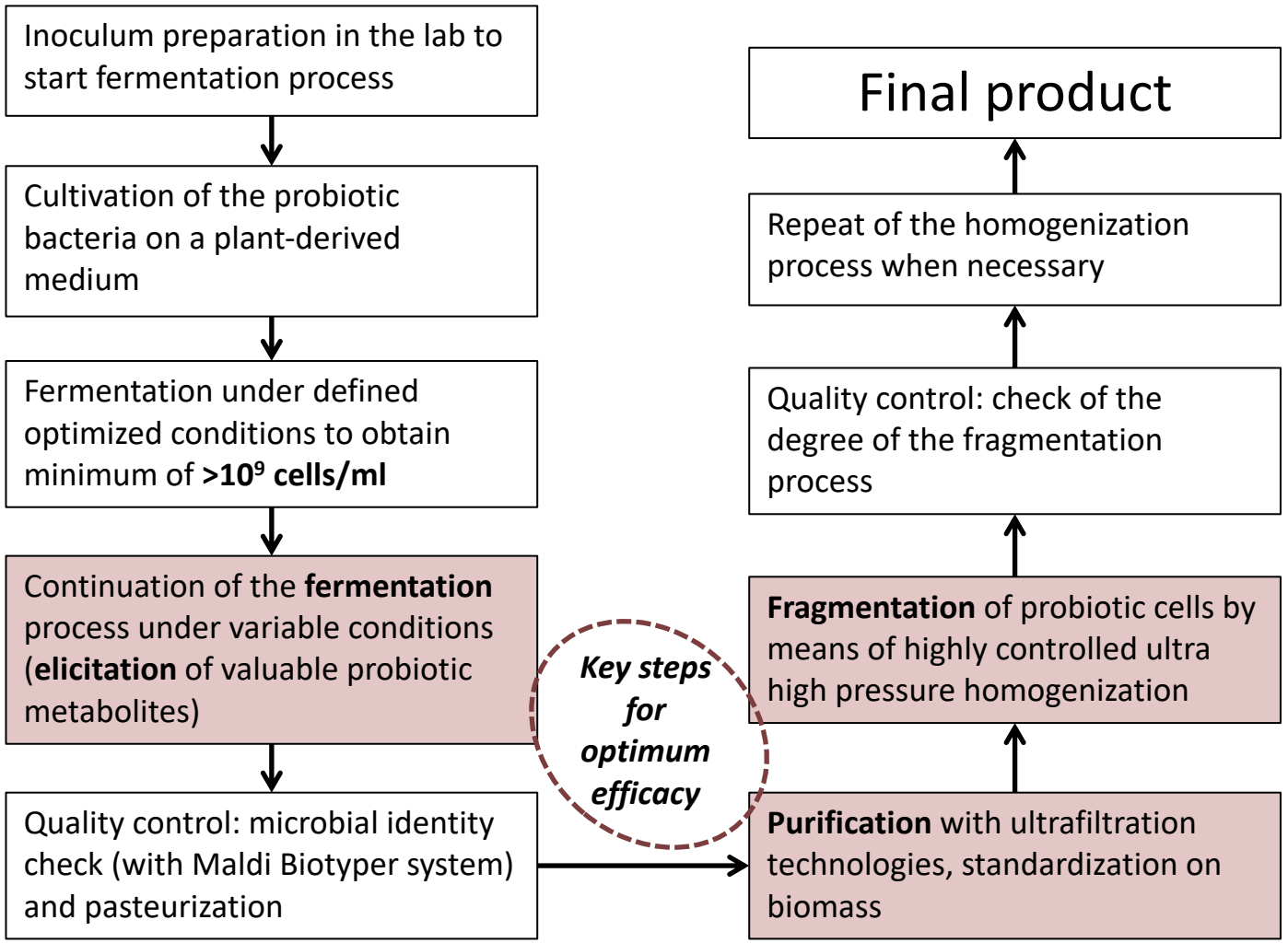


Since the 1970s: 100s of experiments by dozens of scientists (chemists, biotechnologists, biochemists, cell biologists, engineers, microbiologists, computational biologists, etc.)

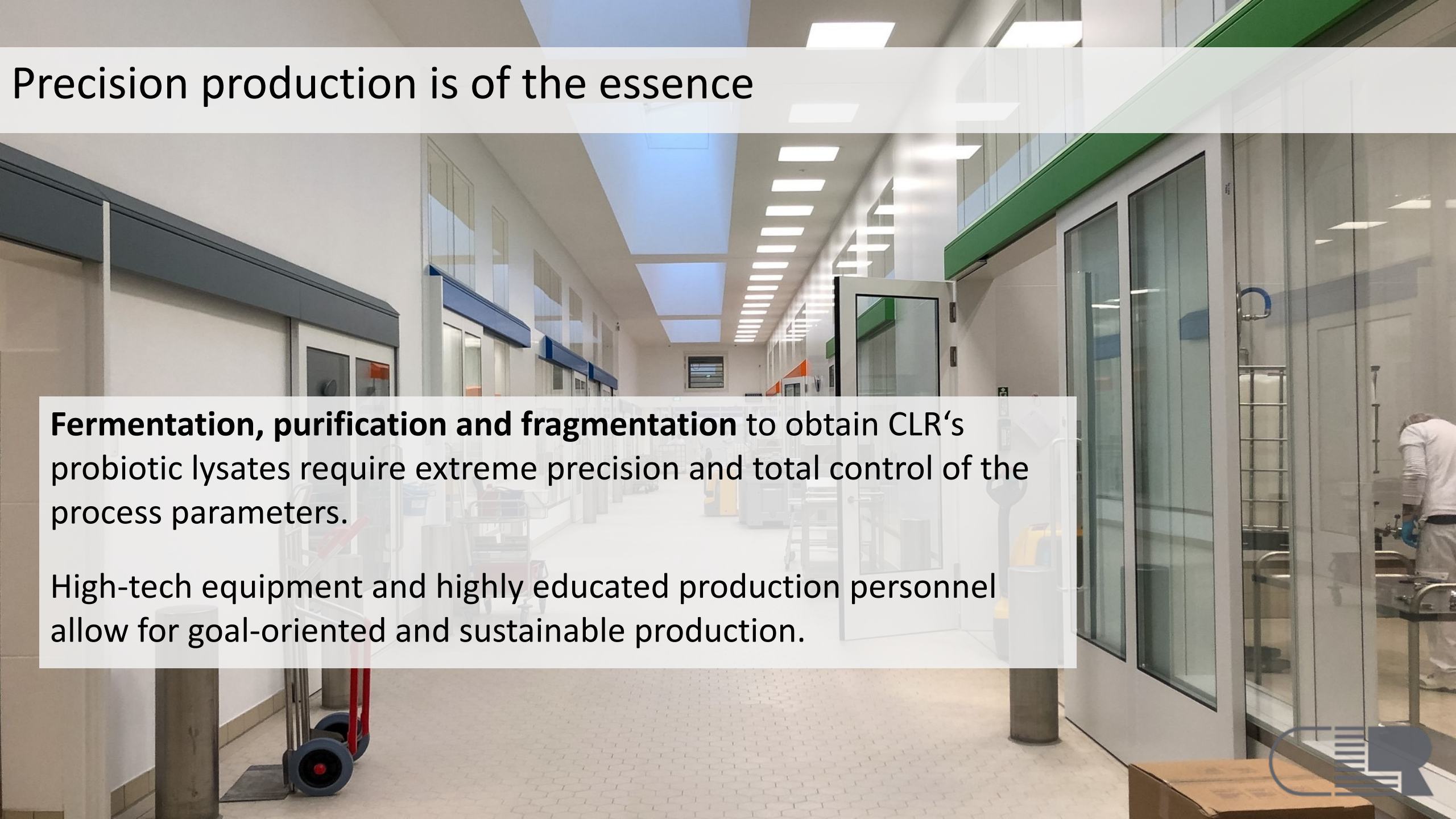
- ✓ Processing: fermentation, purification, fragmentation
- ✓ Chemical analysis
- ✓ Skin cell culture
- ✓ Skin models
- ✓ Clinical studies

Now and into the future

CLR‘s unique expertise: fermentation, purification, fragmentation



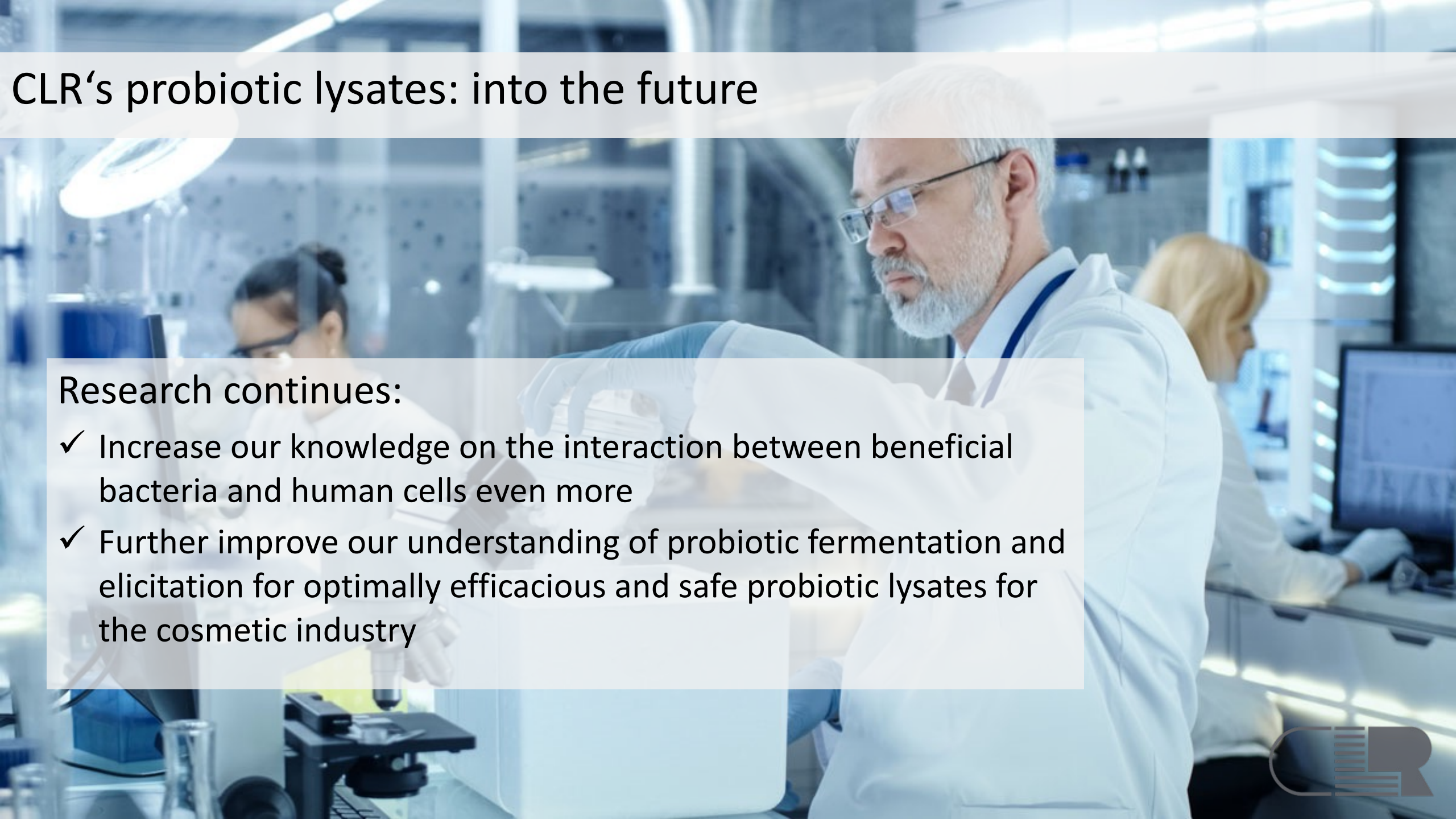
Precision production is of the essence



Fermentation, purification and fragmentation to obtain CLR‘s probiotic lysates require extreme precision and total control of the process parameters.

High-tech equipment and highly educated production personnel allow for goal-oriented and sustainable production.

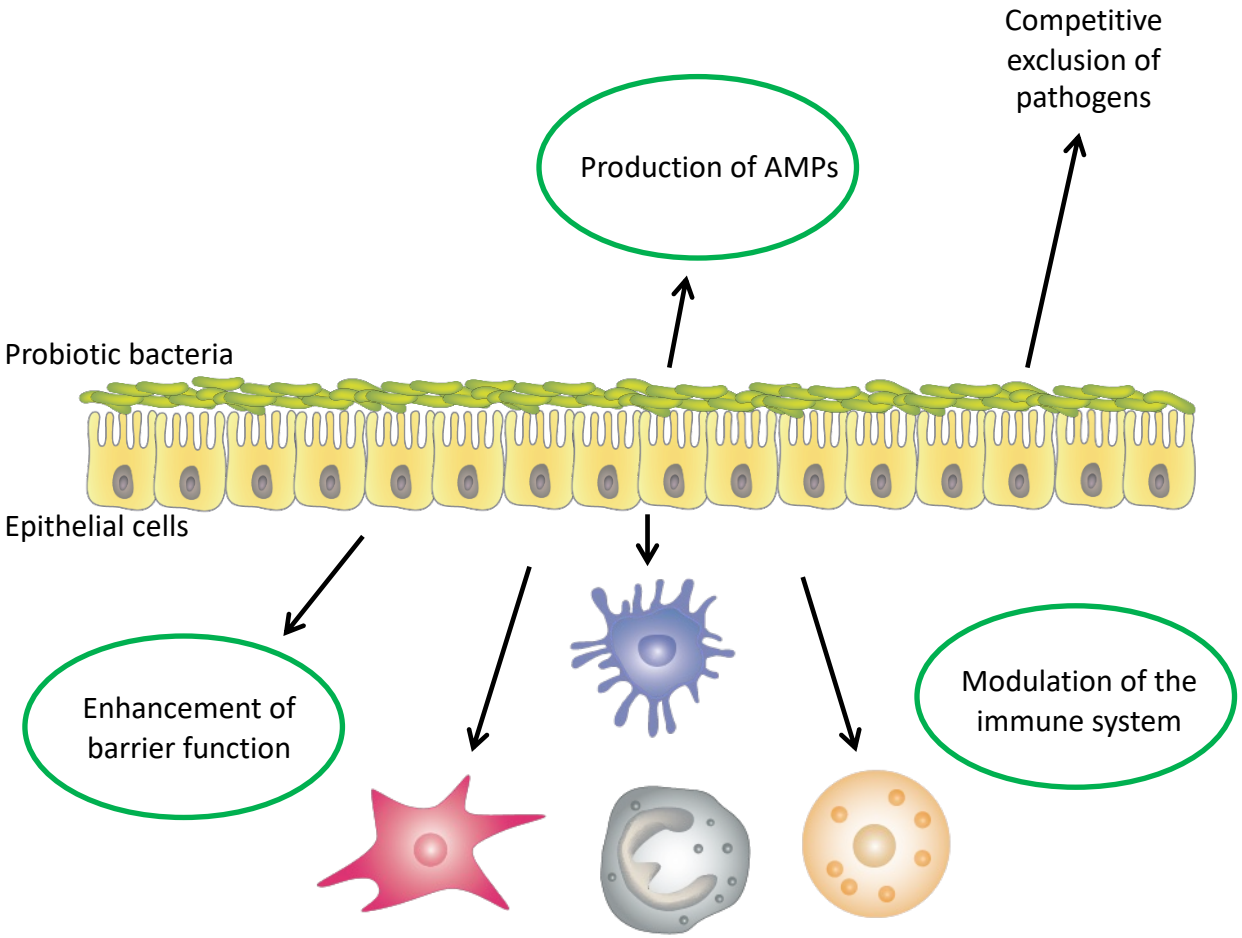
CLR‘s probiotic lysates: into the future



Research continues:

- ✓ Increase our knowledge on the interaction between beneficial bacteria and human cells even more
- ✓ Further improve our understanding of probiotic fermentation and elicitation for optimally efficacious and safe probiotic lysates for the cosmetic industry

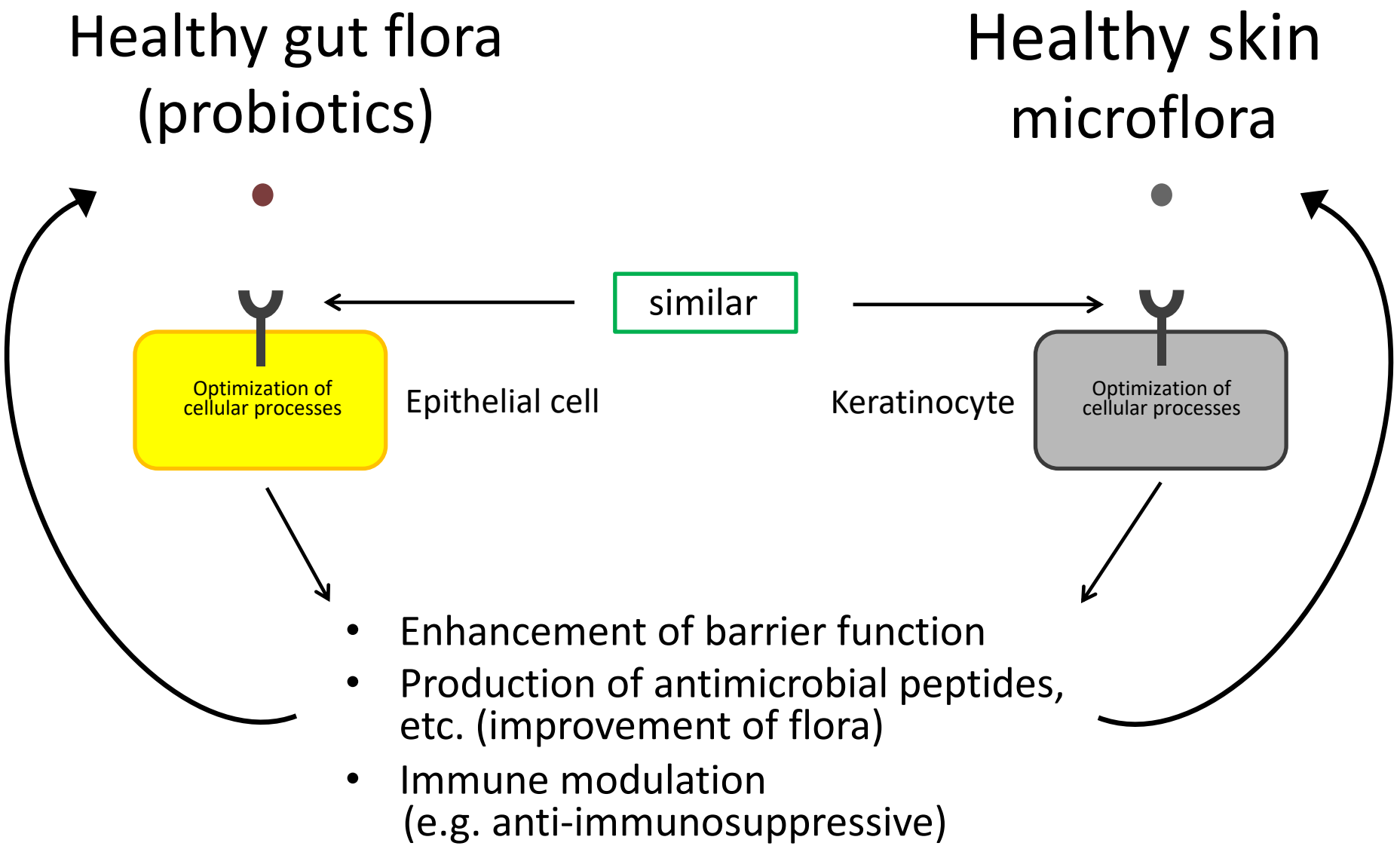
Probiotic bacteria in our gut



‚Good' bacteria on the skin play a similar role

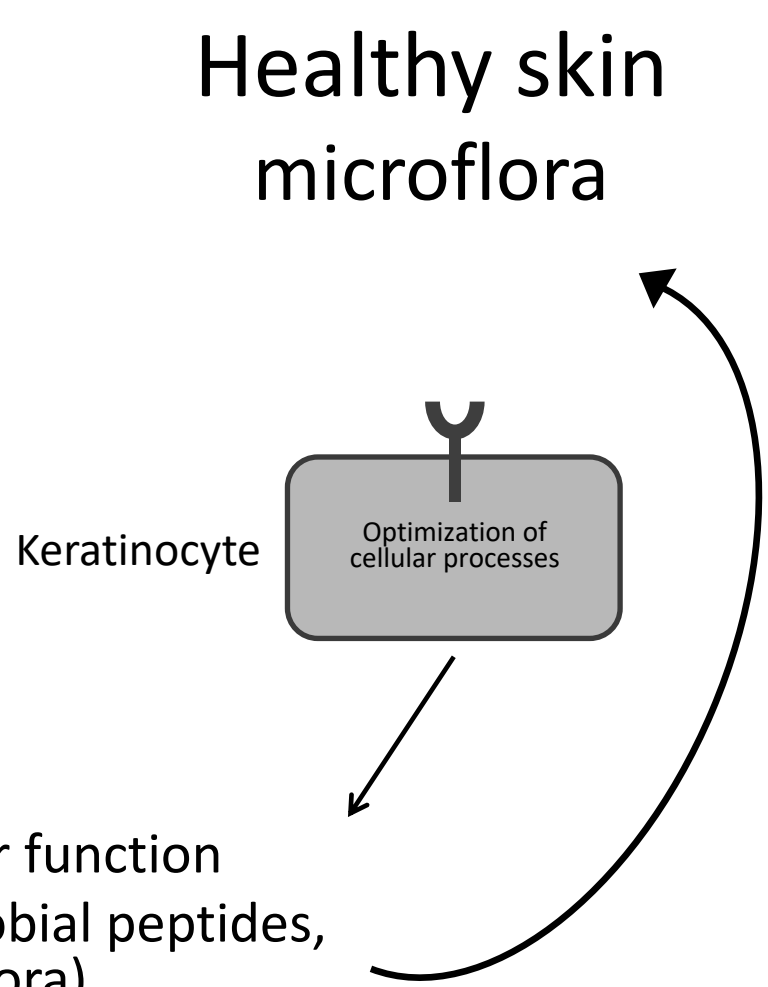
Mechanism: biomolecules and receptors

Probiotic and skin bacteria



Probiotic and skin bacteria – probiotic lysates

- **Lactococcus Ferment Lysate**
  - **ProRenew Complex CLR™**
- **Bifida Ferment Lysate**
  - **Repair Complex CLR™ PF**
  - **G+C Complex CLR™**



- Enhancement of barrier function
- Production of antimicrobial peptides, etc. (improvement of flora)
- Immune modulation (e.g. anti-immunosuppressive)

ProRenew Complex Complex CLR™

Psotbiotic support for skin and its microbiota

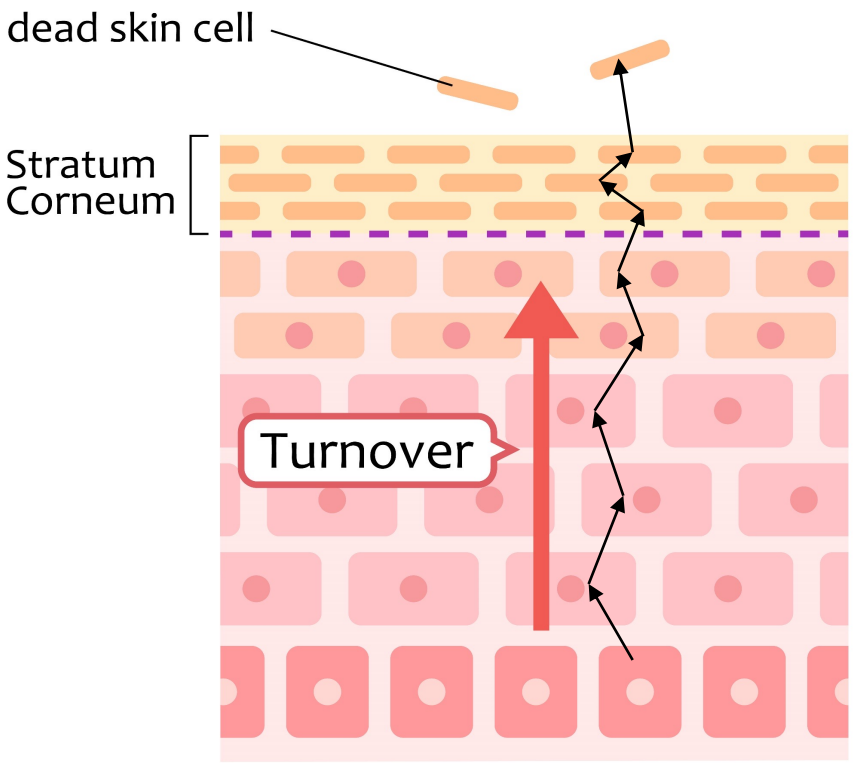
ProRenew Complex CLR™

- Obtained from a lysate of *Lactococcus lactis*
- Biotechnologically obtained suspension from a probiotic lactic acid producing bacterium
- Contains cytoplasm and cell wall fragments which increase the immunocompetence of skin cells

First: The Skin

Focus on Epidermis

Skin's Primary Task: Task #1



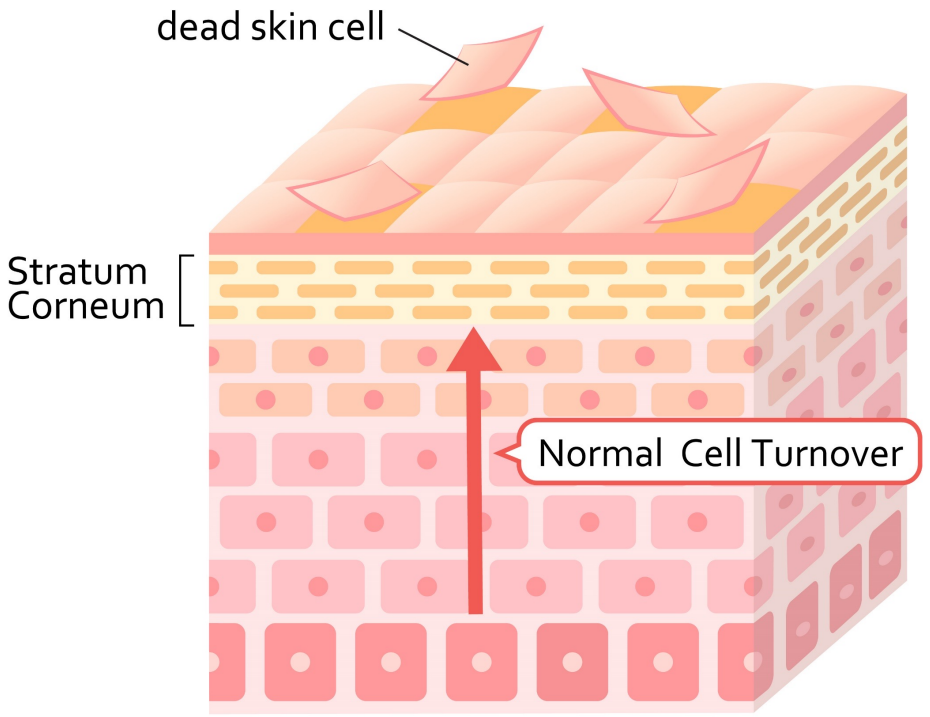
Task #1: Build and maintain a physical barrier function (Stratum Corneum)

Essential: Constant renewal

Epidermal turnover: appr. 4 weeks

Per day: 1.5 g of dead skin cells is shed

Epidermal Turnover: Essential Outcomes

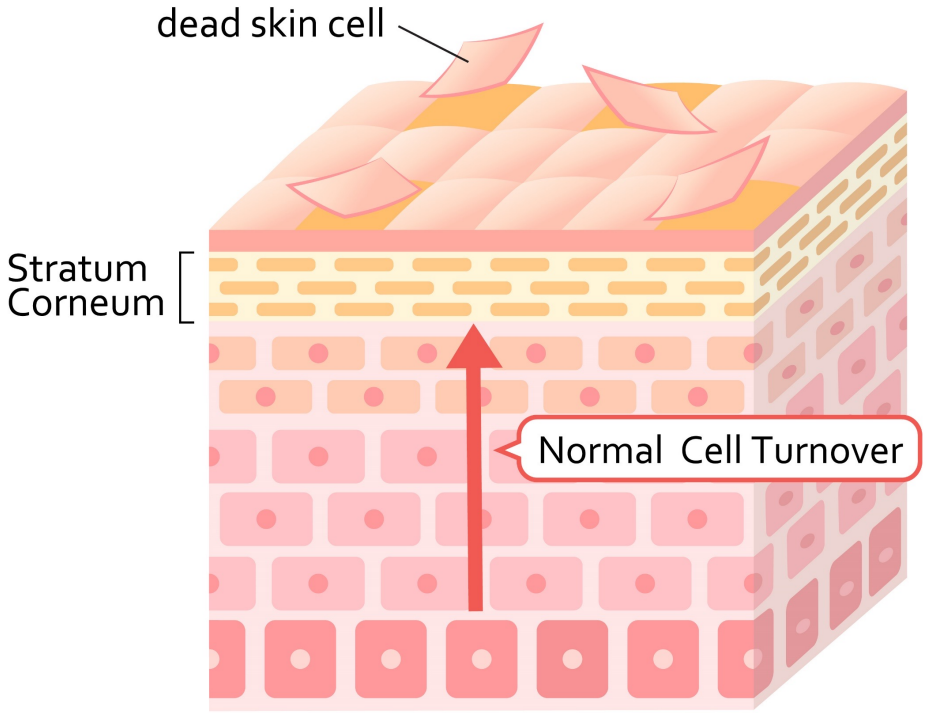


Epidermal Cell Turnover:

- Extremely important
- Extremely well-regulated and complex
- Extremely vulnerable ← !!!!!!!

Epidermal Turnover: Essential Outcomes

Strong barrier | Smooth | Attractive | Hydrated | Youthful | Radiant



Epidermal Cell Turnover:

- Extremely important
- Extremely well-regulated and complex
- Extremely vulnerable ← !!!!!!!

Epidermal Turnover: Essential Outcomes

Strong barrier

Smooth

Attractive

Hydrated

Youthful

Radiant

Rough

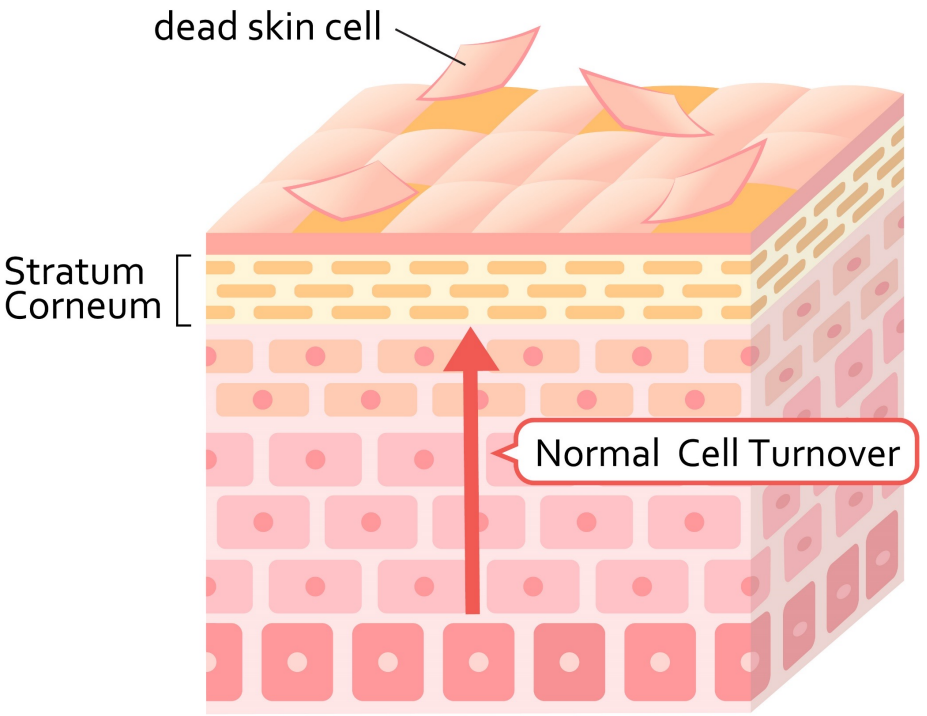
Weak barrier

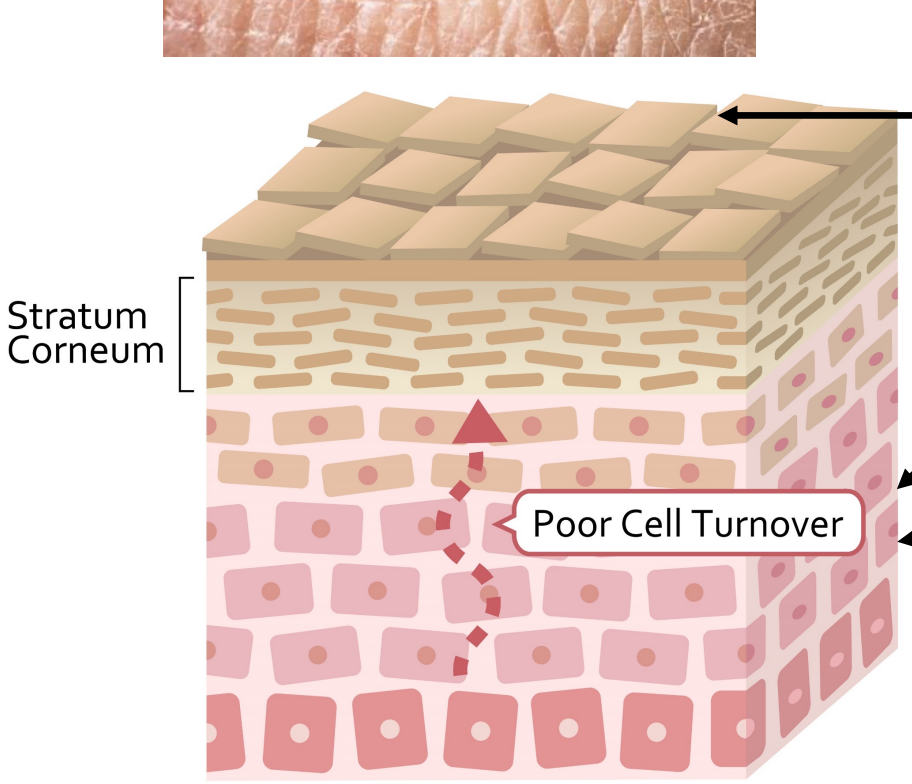
Dry

Unattractive

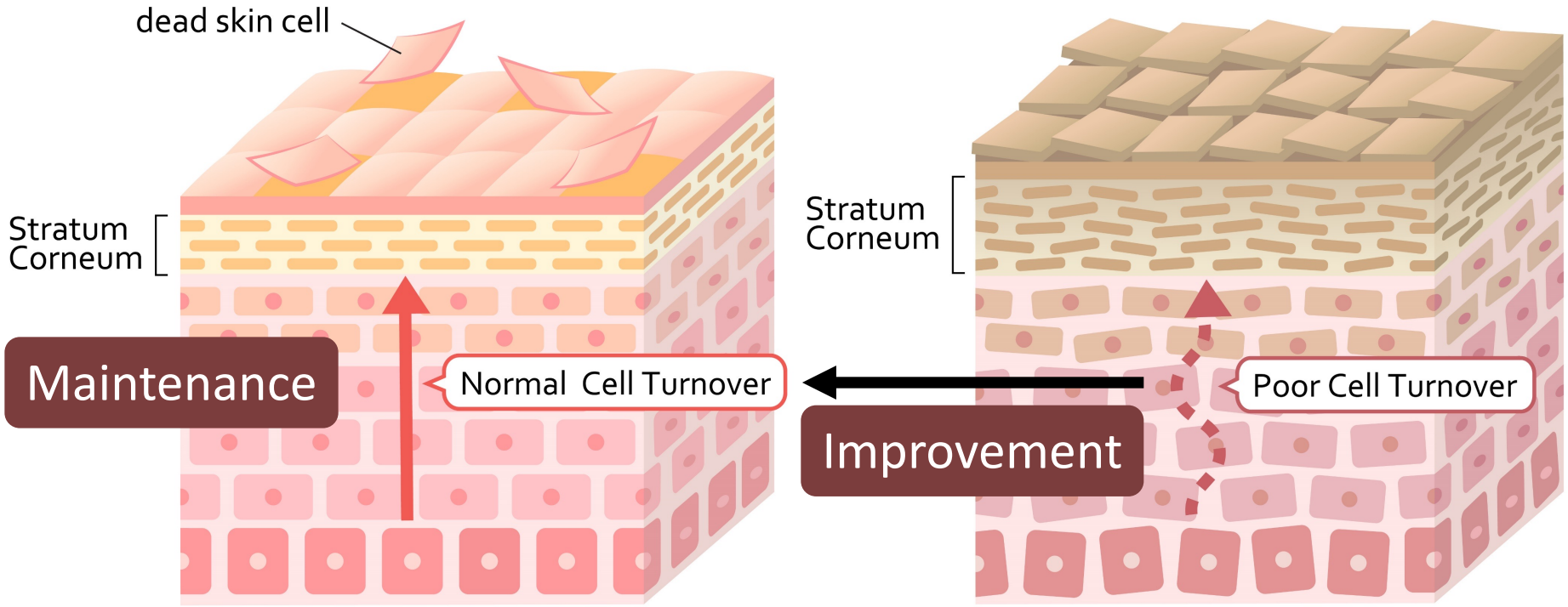
Dull

Old





Epidermal Turnover: Primary Goals

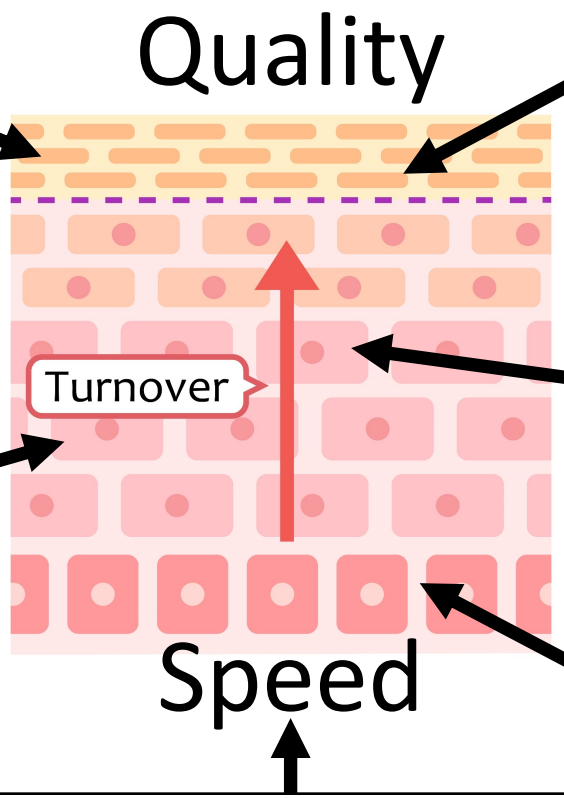


In Vitro Activity on Epidermal Turnover - Summary

Improvement of barrier function, epidermal skin model: 17.0% better than control

Gene expression (fold increase):

KLK5 (Kalikkrein5):	2.3
KLK7 (Kalikkrein 7):	2.4
OCLN (Occludin):	2.7
IVL (Involucrin):	3.8
FLG (Filaggrin):	2.9
DSG1 (Desmoglein 1):	3.4
DSC1 (Desmocollin 1):	3.1



Clear acceleration of epidermal turnover, epidermal skin model

Increase in production of antimicrobial peptides, keratinocytes:

Cathelicidin (LL-37):	185.0%
β Defensin-1:	147.7%

Histological evaluation (protein expression), epidermal skin model.

Increase in:

- Loricrin
- Transglutaminase 1
- Profilaggrin/Filaggrin
- Caspase 14

Increase in keratinocyte cohesion:

6.5 – 8.0%

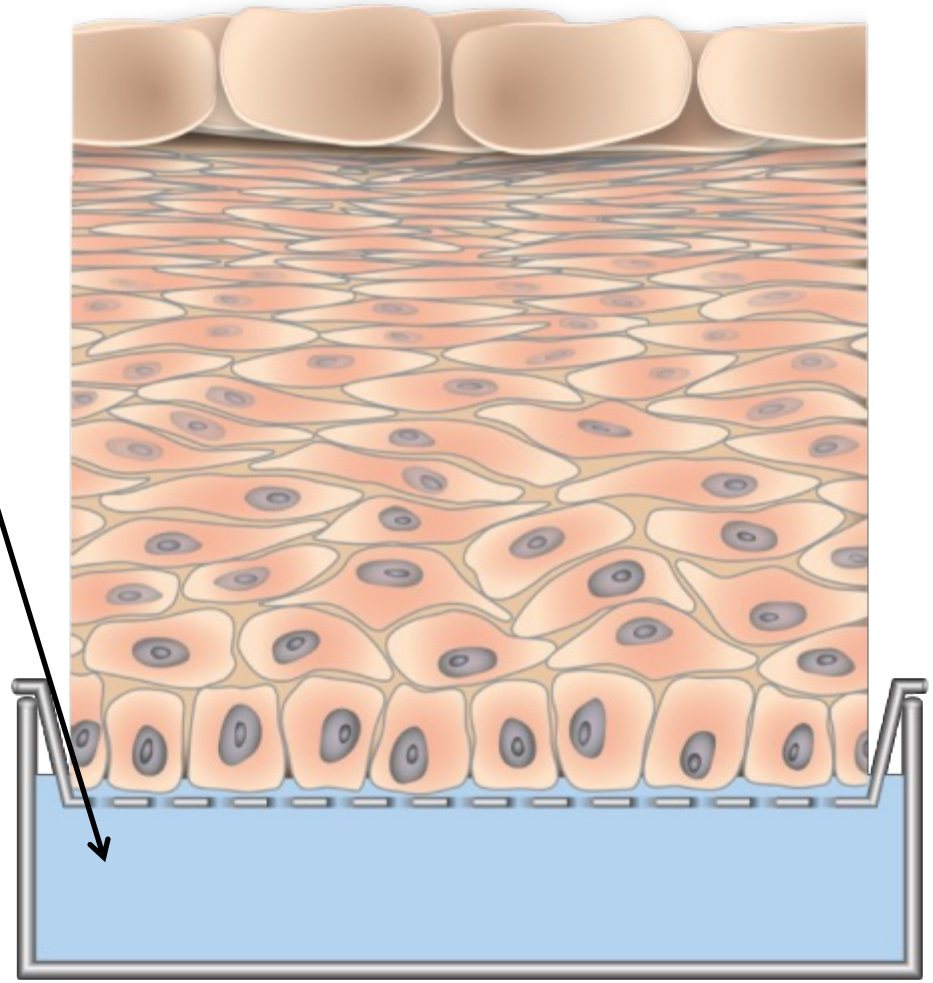
Influence on epidermal growth

14 day growth in presence of ProRenew Complex CLR™ (in medium, added from day 4)

Histological evaluation of differentiation markers:

- Loricrin
- Transglutaminase 1 (TG1)
- Profilaggrin and Filaggrin
- Caspase 14
- Stratum Corneum maturity

Evaluation of barrier function (epiCS, CellSystems GmbH, Germany)

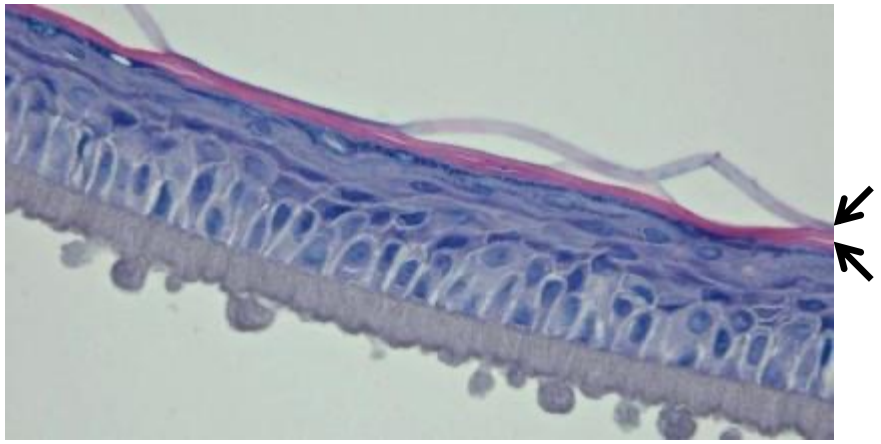


Histological evaluation: Stratum Corneum maturity

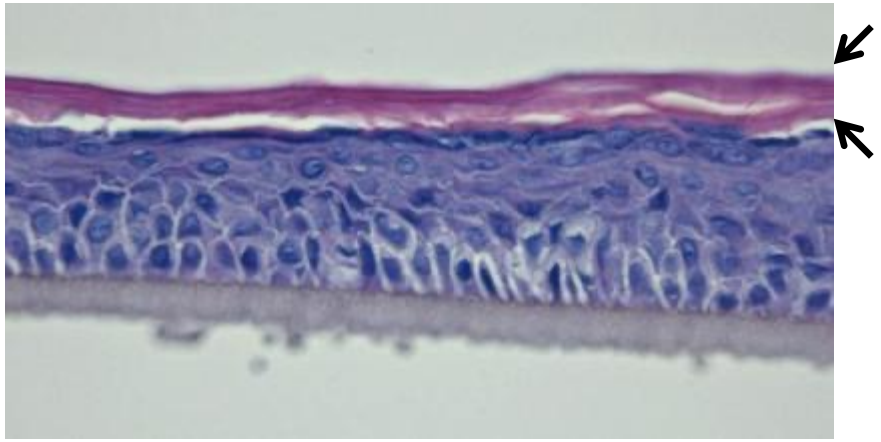
Day 10:

Epidermal skin model treated with ProRenew Complex CLR™ clearly shows a thicker Stratum Corneum as compared to control.

(Haematoxylin–Eosin stain, magnification: 400X)

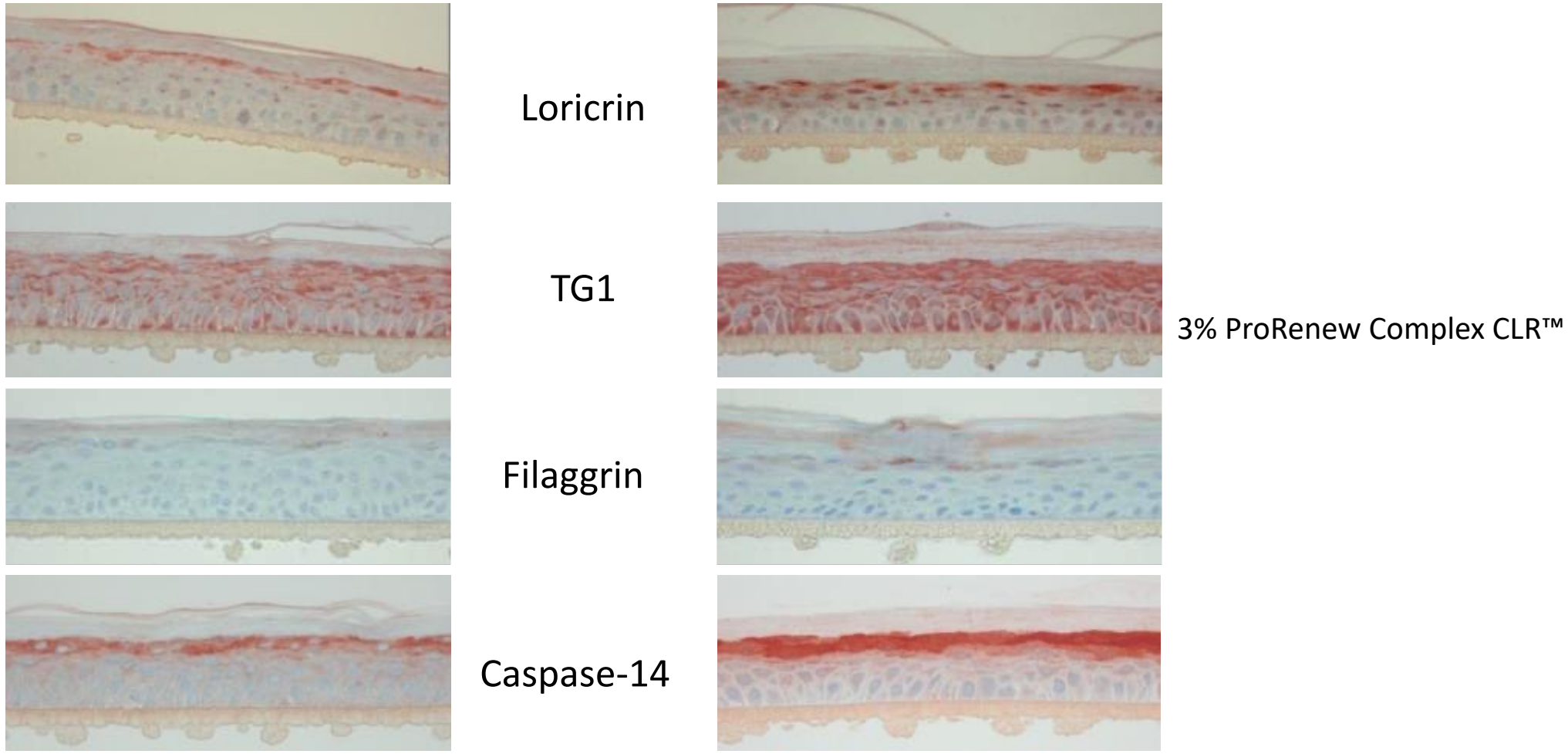


Control



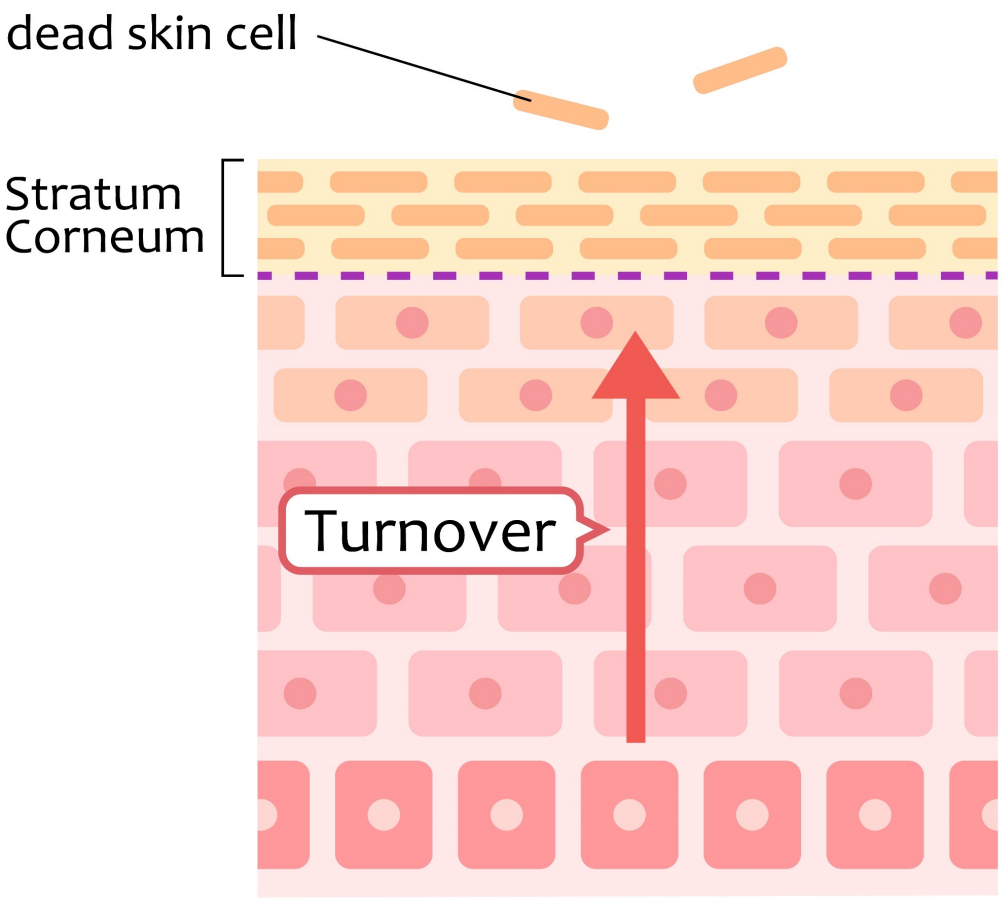
3% ProRenew Complex CLR™

Histological evaluation: Summary



In vivo studies on human volunteers

Parameters of “Youthful Skin Health”



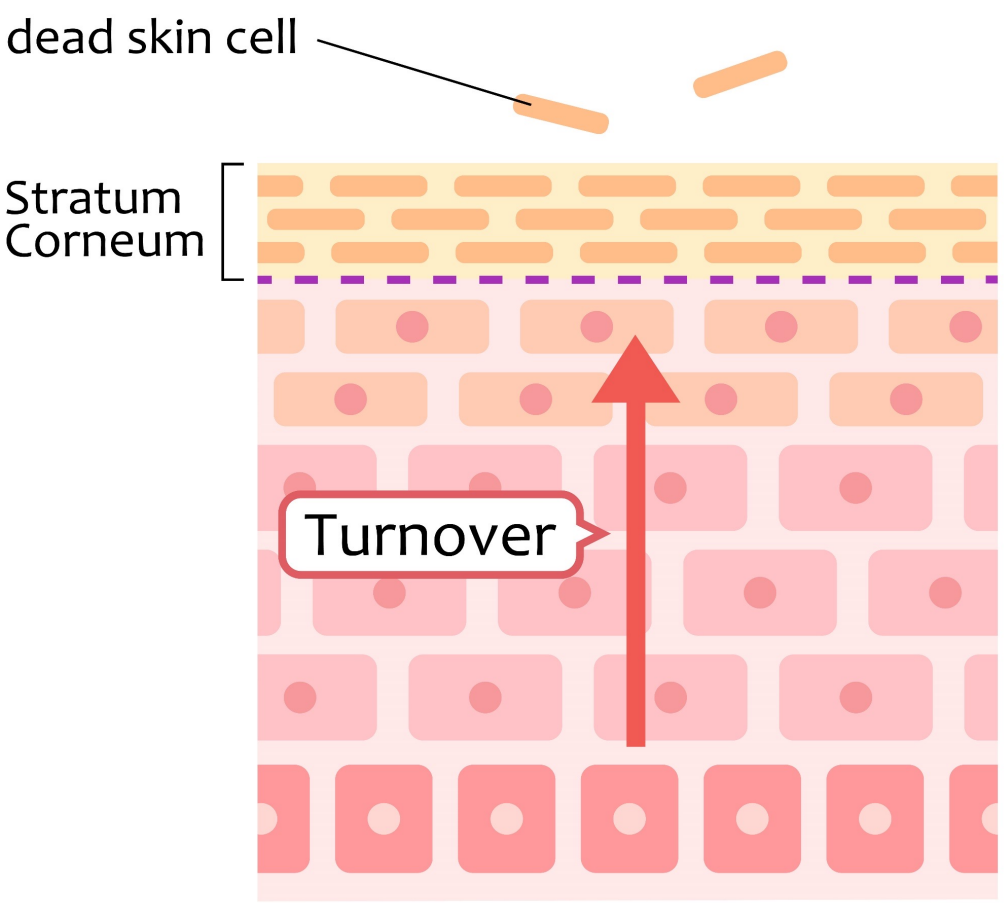
Desquamation:

The effective shedding of dead skin cells

Epidermal Turnover:

1. Speed
2. Quality

Parameters of “Youthful Skin Health”



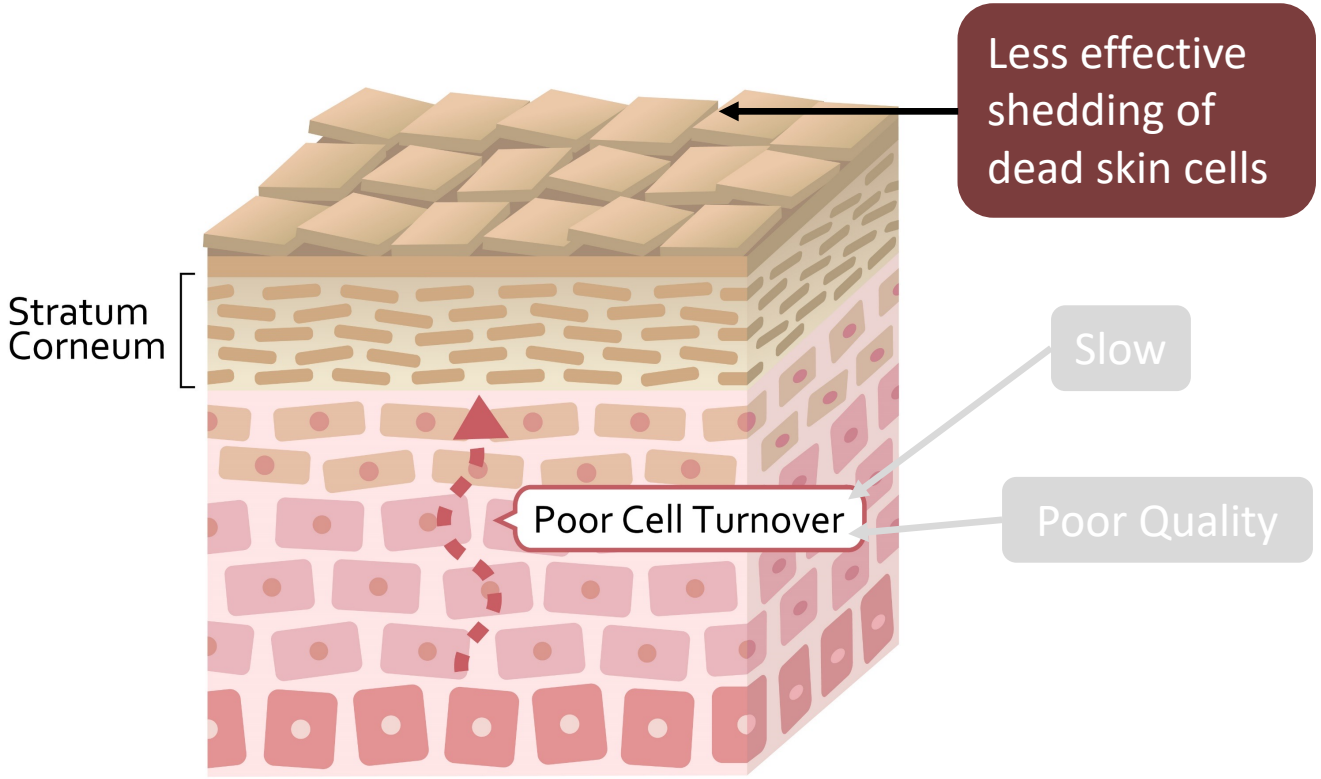
Desquamation:

The effective shedding of dead skin cells

Epidermal Turnover:

1. Speed
2. Quality

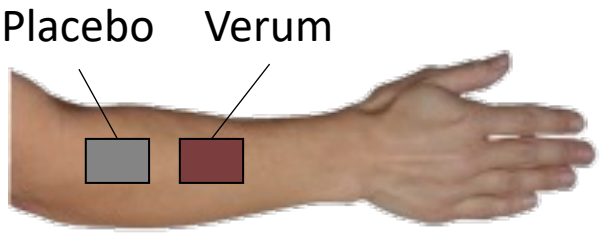
Parameter to Improve: Desquamation



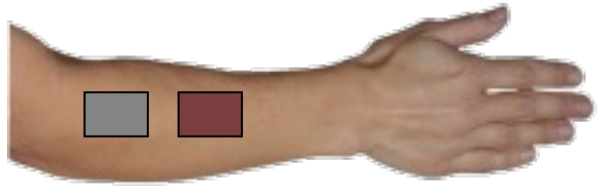
ProRenew Complex CLR™

Determination of effectiveness of desquamation

18 female volunteers (52-70 yrs old), outer forearm

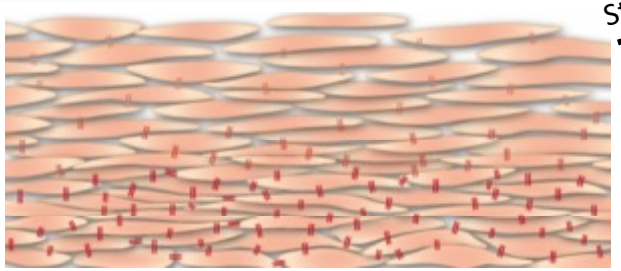


Application of test products, 42 days, twice daily



Day 43: tape stripping
Total of 5 strips

Strips 1-5, together



Determination of total protein mass on strips (*higher = more dead skin cells = better desquamation*)
→ Conclusions on effectiveness of desquamation

ProRenew Complex CLR™

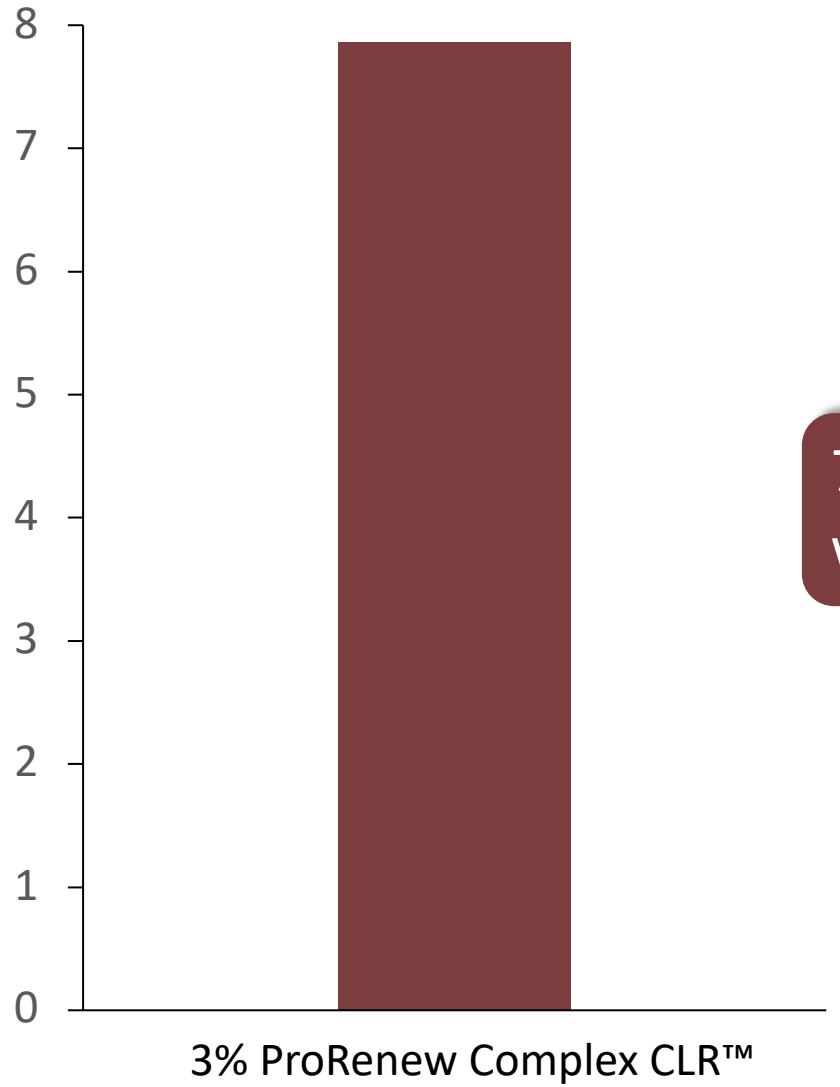
Influence on Desquamation

Effectiveness of desquamation (%)

On designated areas on the outer forearm of 18 volunteers (52-70 yrs old, average 62.4) placebo and corresponding formulation containing 3% ProRenew Complex CLR™ were applied twice daily, for 42 days.

On day 43 treated areas were tape stripped totaling 5 strips per area. Total protein mass on strips 1-5 was quantified.

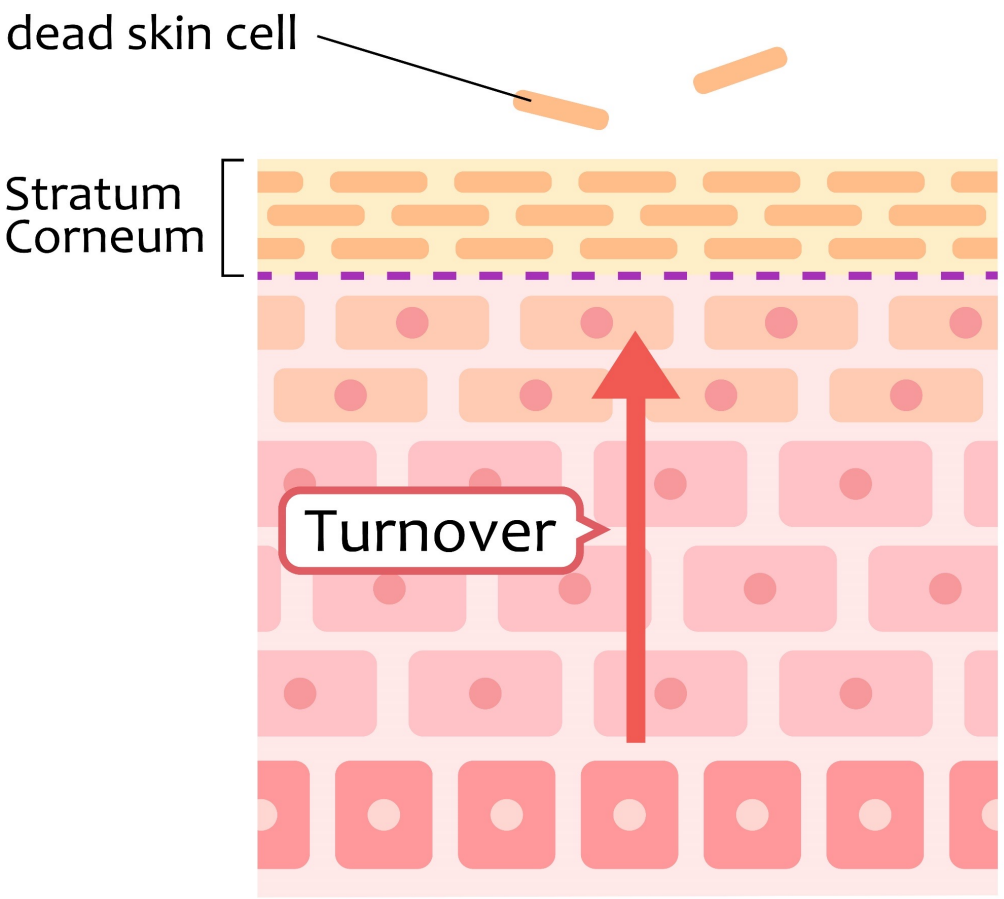
Protein mass on strips of placebo-treated skin was set at 0%.



7.9 % better than with Placebo

ProRenew Complex CLR™

Parameters of “Youthful Skin Health”



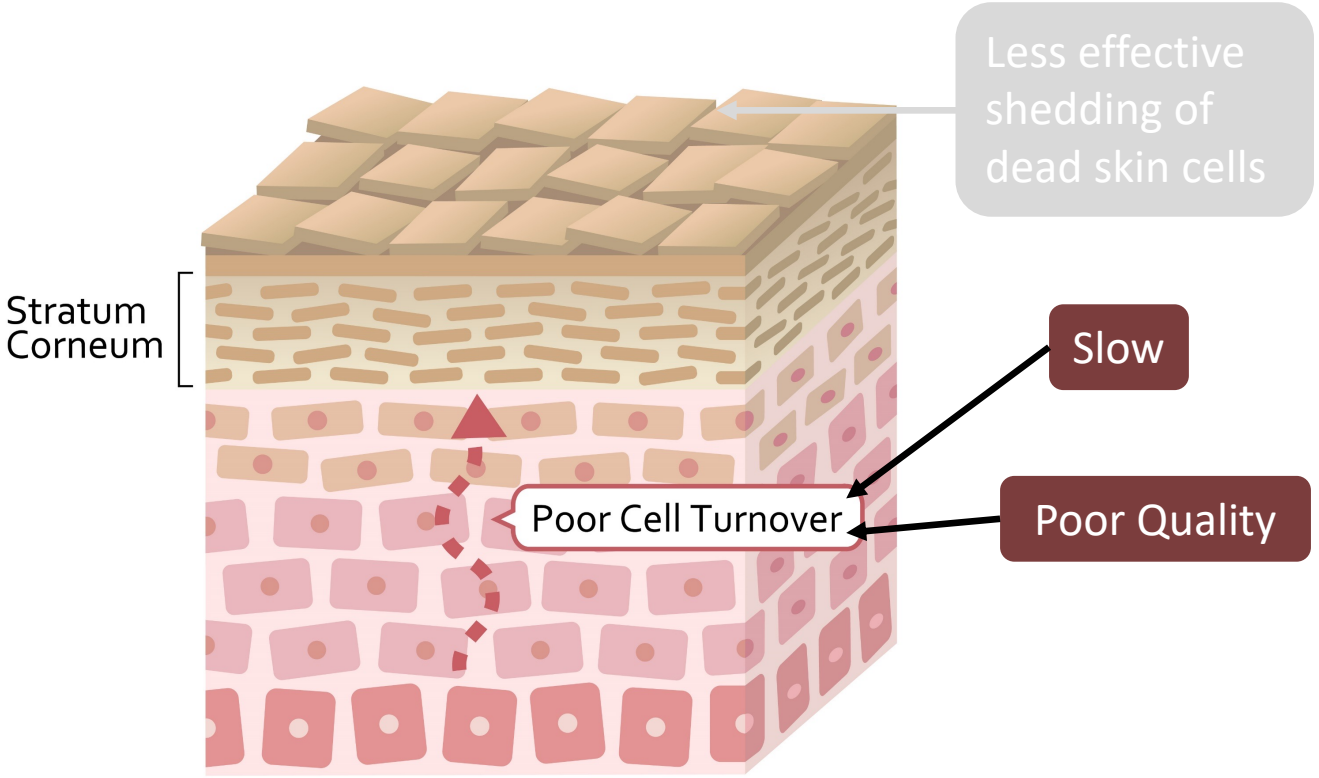
Desquamation:

The effective shedding of dead skin cells

Epidermal Turnover:

1. Speed
2. Quality

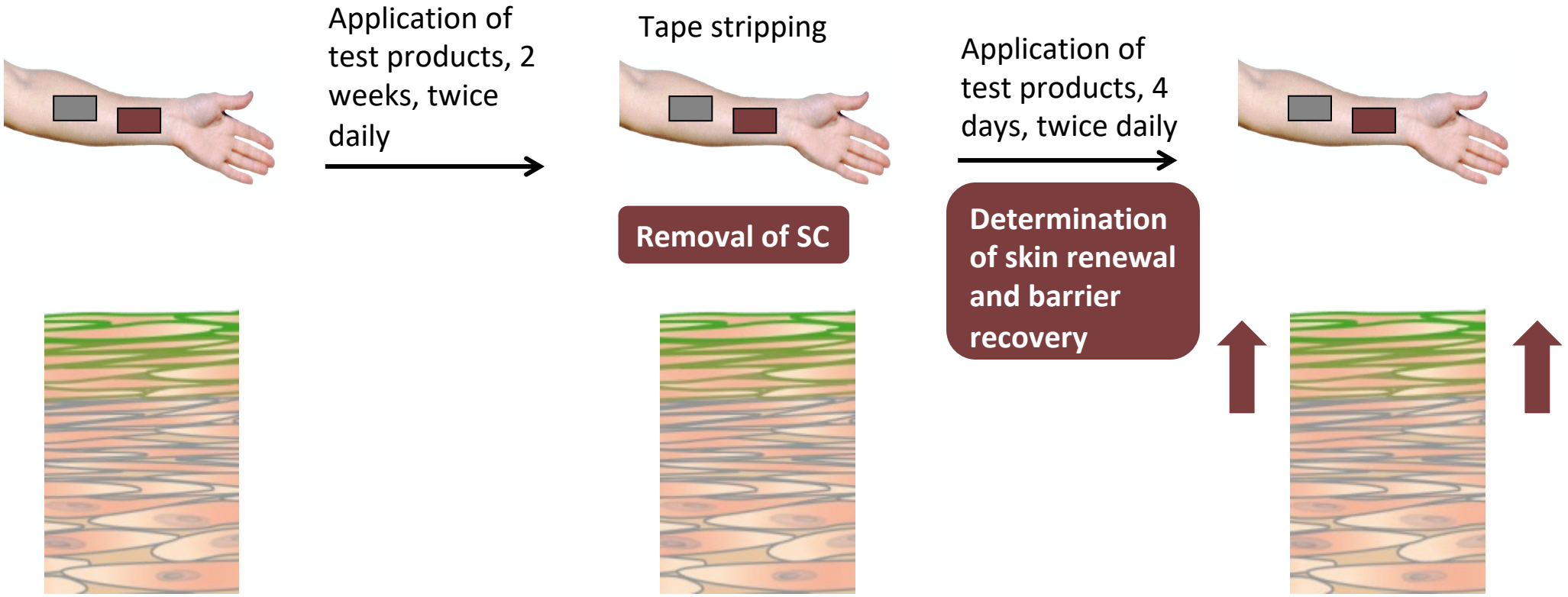
Parameters to Improve: speed and quality of renewal



ProRenew Complex CLR™

Skin renewal and barrier recovery – Experimental design

5 female volunteers (47 - 63 years old), inner forearm



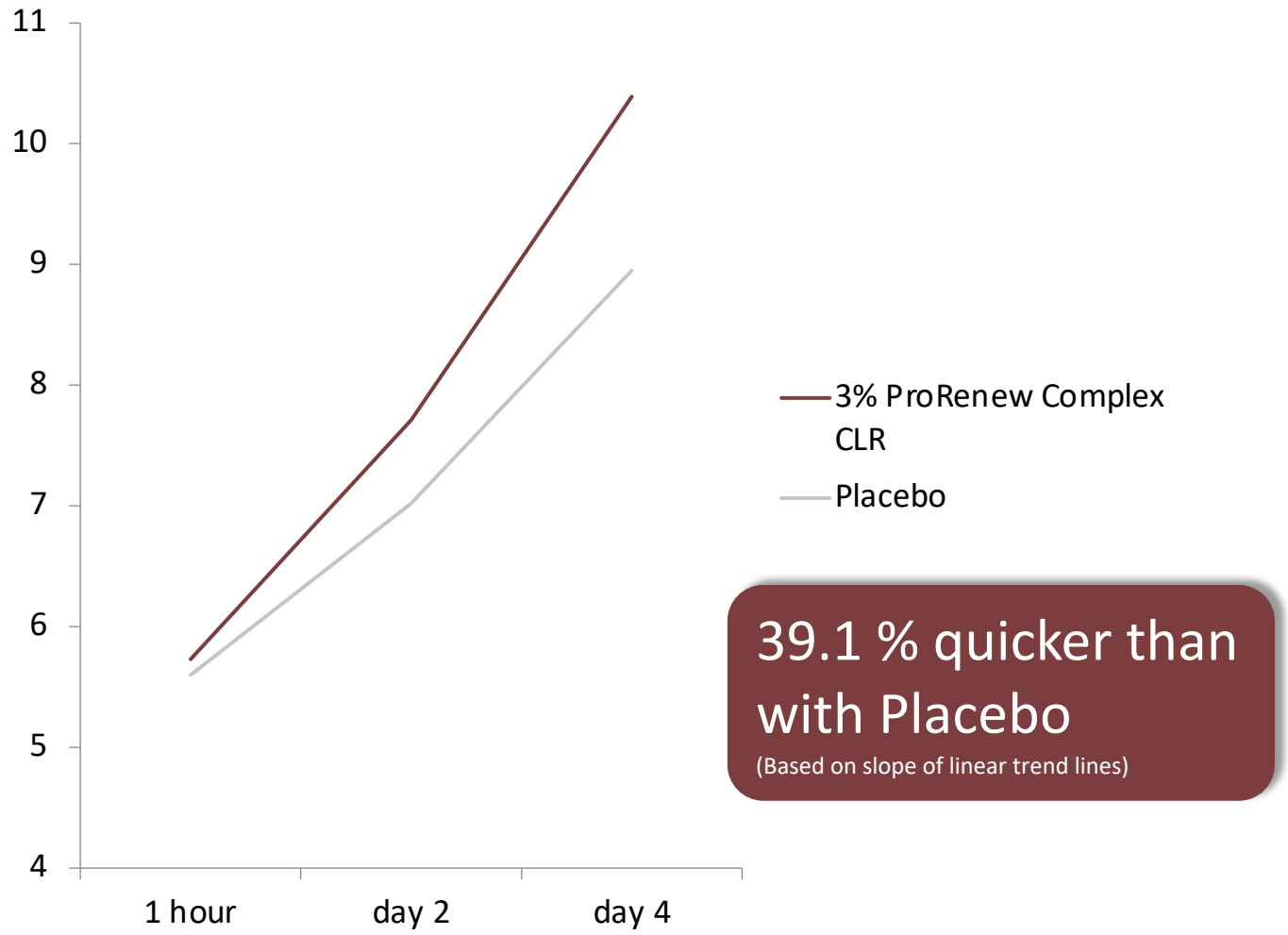
ProRenew Complex CLR™

Acceleration of skin turnover

SC thickness (µm)

SC thickness was determined one hour after tape stripping, 2 and 4 days after tape stripping.

(method: Vivascope® 1500, Lucid Inc., Rochester, NY, USA)



ProRenew Complex CLR™

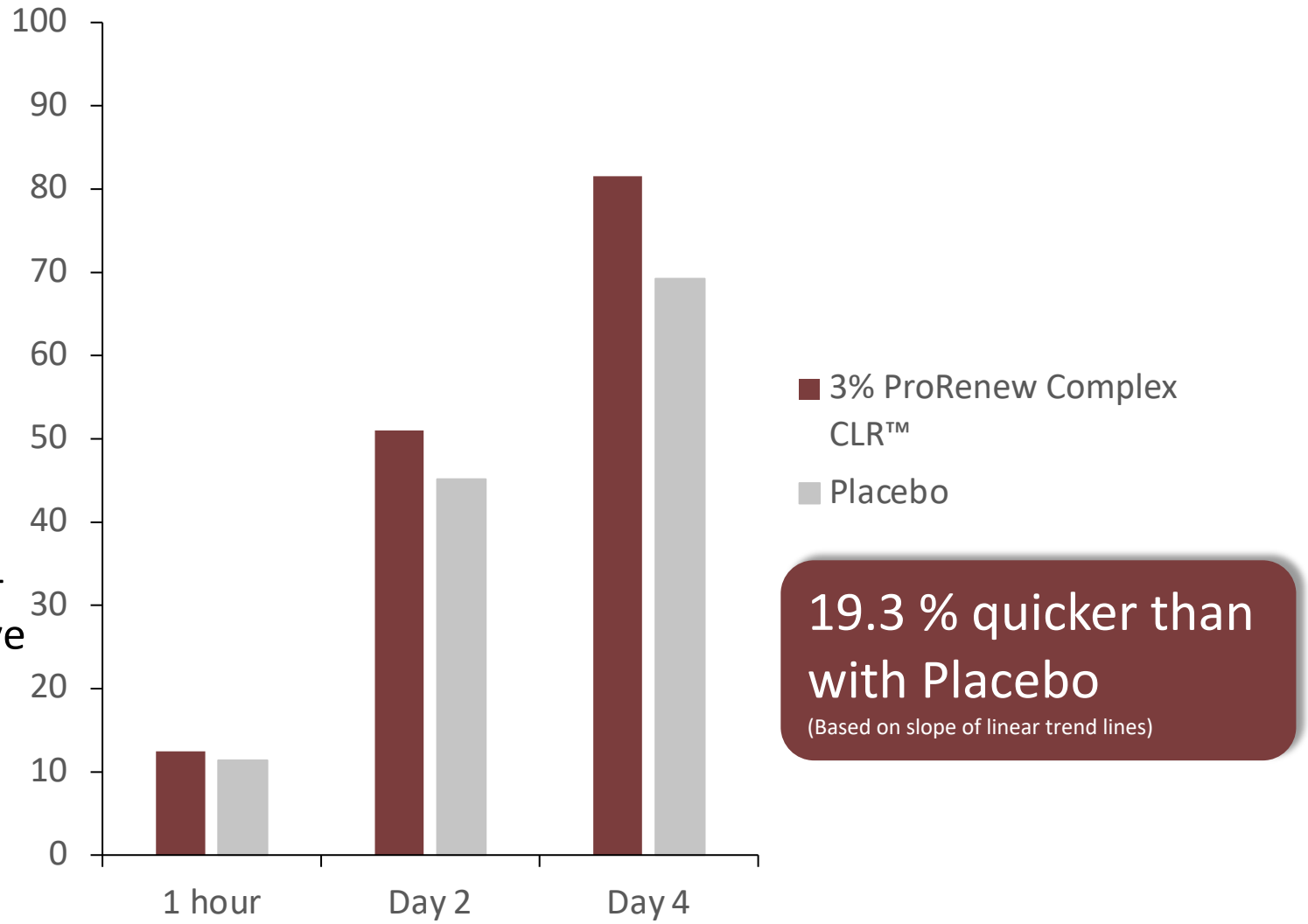
Stimulation of barrier recovery

Barrier recovery (%)

TEWL was measured one hour, 2 and 4 days after tape stripping.

Method: Tewameter TM 210, Courage & Khazaka, Germany

Positive control: skin which was not tape-stripped and not treated, at the respective time points. Set at 100%.



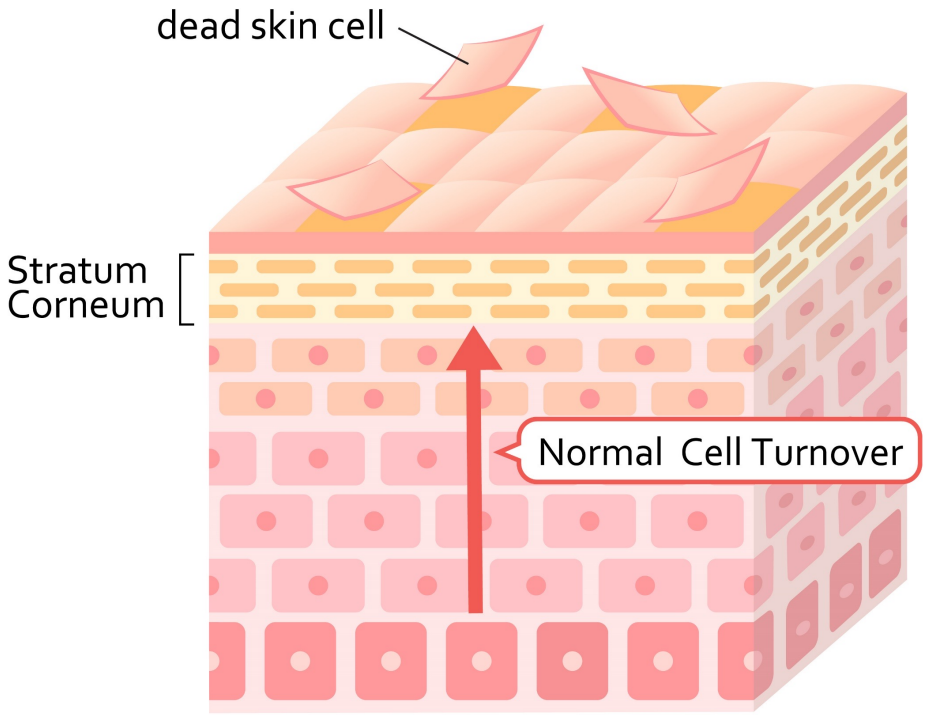
ProRenew Complex CLR™

Now: The Skin Microbiome

Short Recap

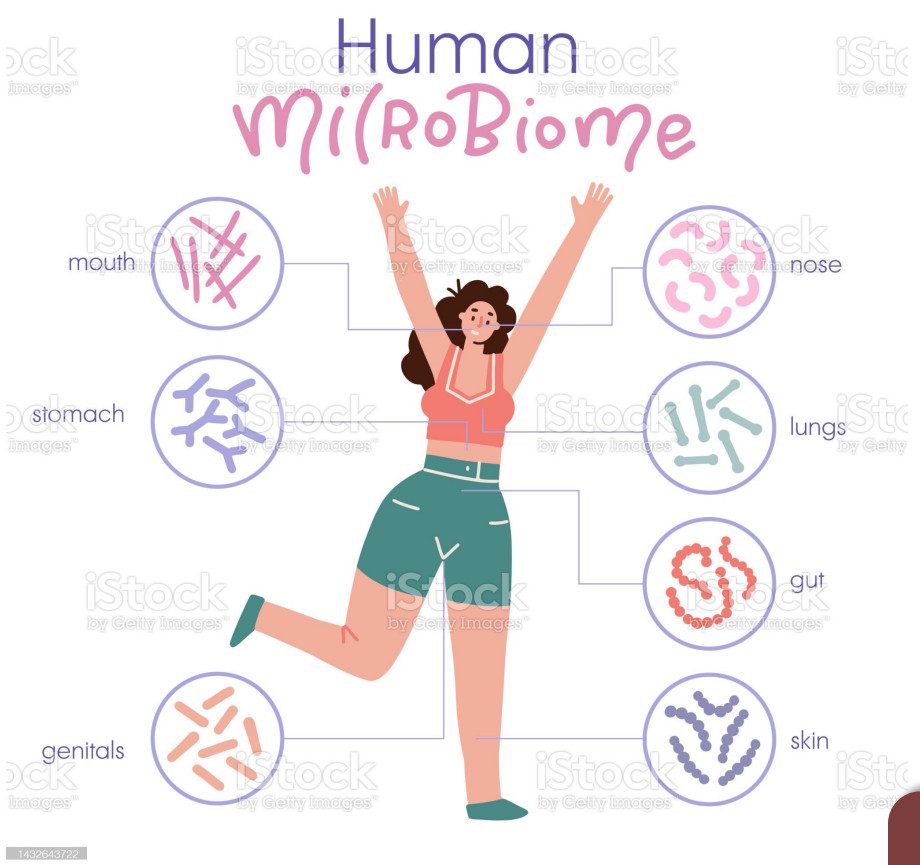
- Strong barrier
- Smooth
- Attractive
- Hydrated
- Youthful
- Radiant

Skin Microbiome:
balance, support, protection



How????

The Skin Microbiome

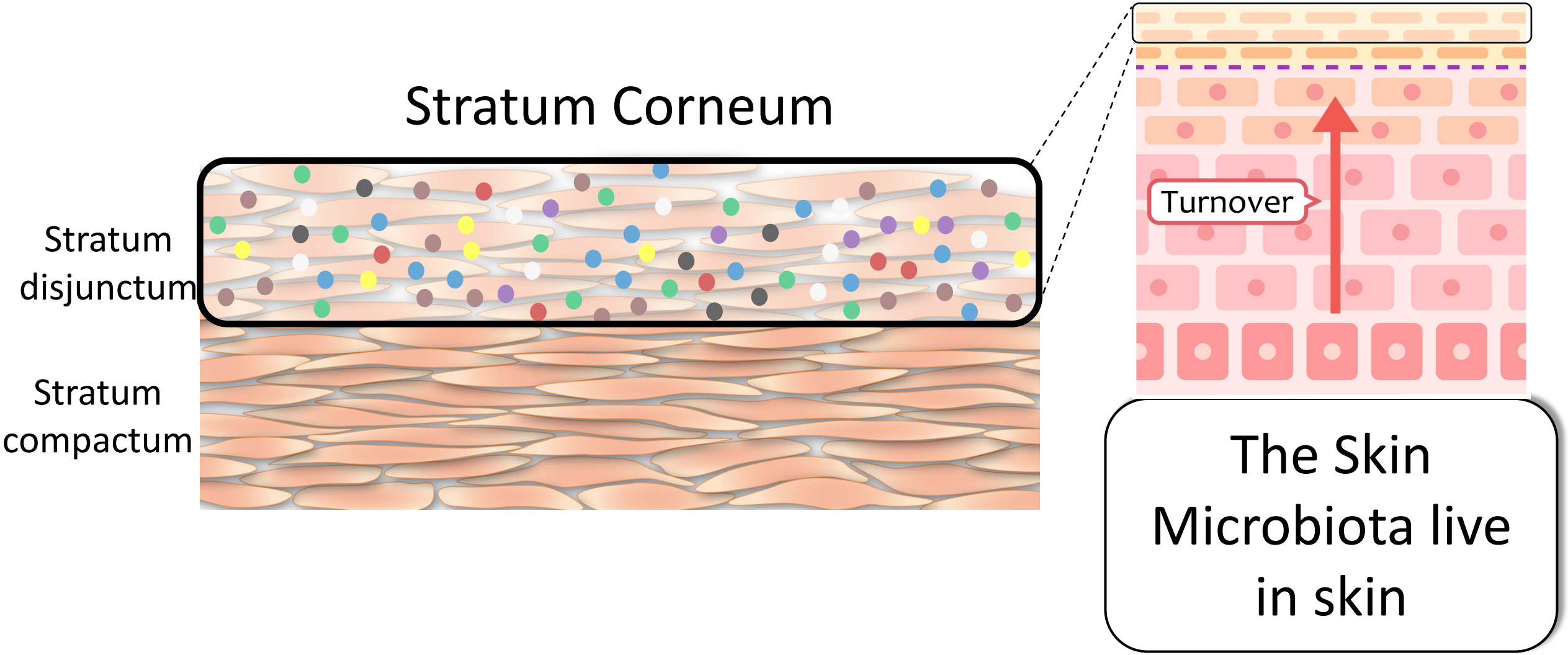


The Skin Microbiome

- ✓ Our skin (25 m^2) has up to a billion microbes per square centimeter
- ✓ Our skin is an ecosystem: Intensive interaction between microbial and skin cells
- ✓ Critical for the quality and healthy functionality of our skin
- ✓ A symbiotic relationship

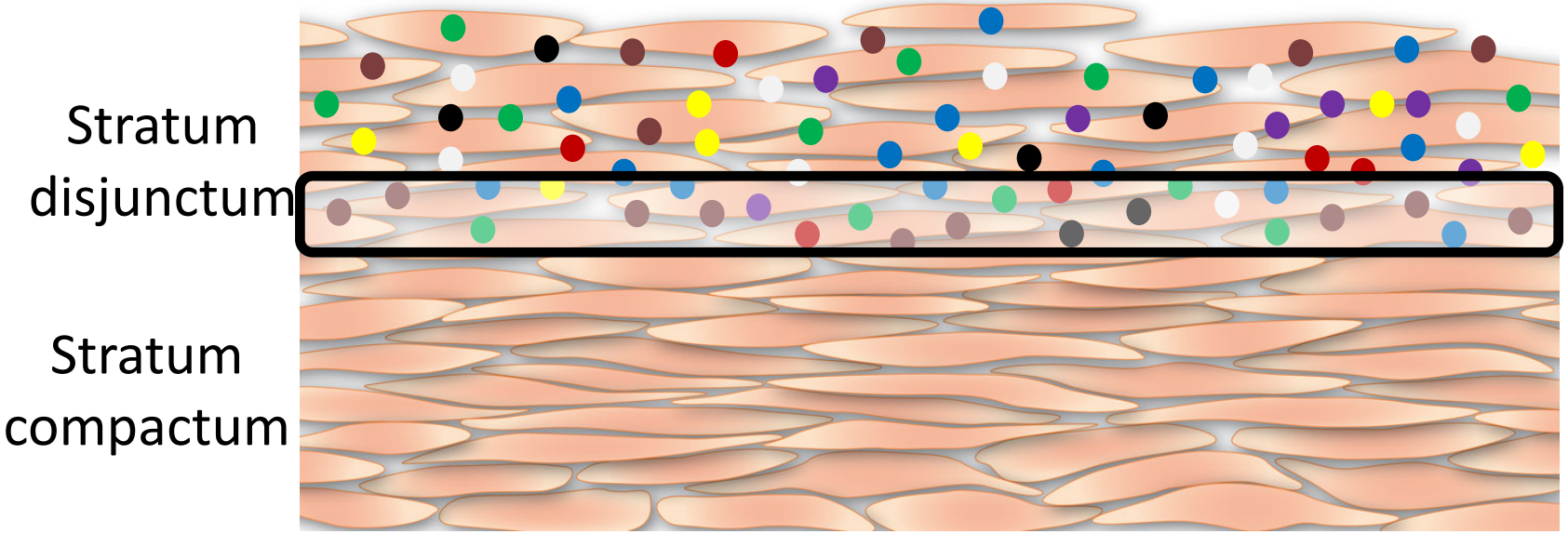
Here too: “The Environment Selects”. The composition of the skin microbiome is dictated by the local environment

The Environment Selects: Where?



The Skin Microbiota live in skin

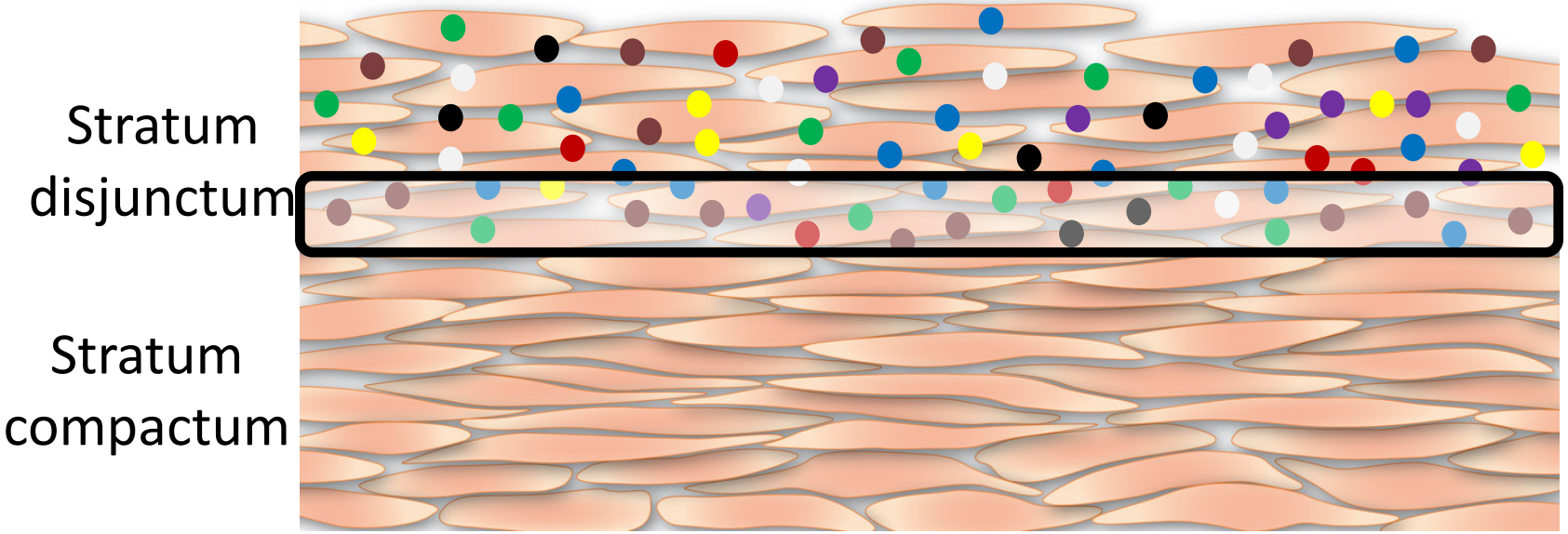
The Skin Microbiota thrive inside the Skin



Microbiota grow and proliferate in the deeper layers of the Stratum disjunctum

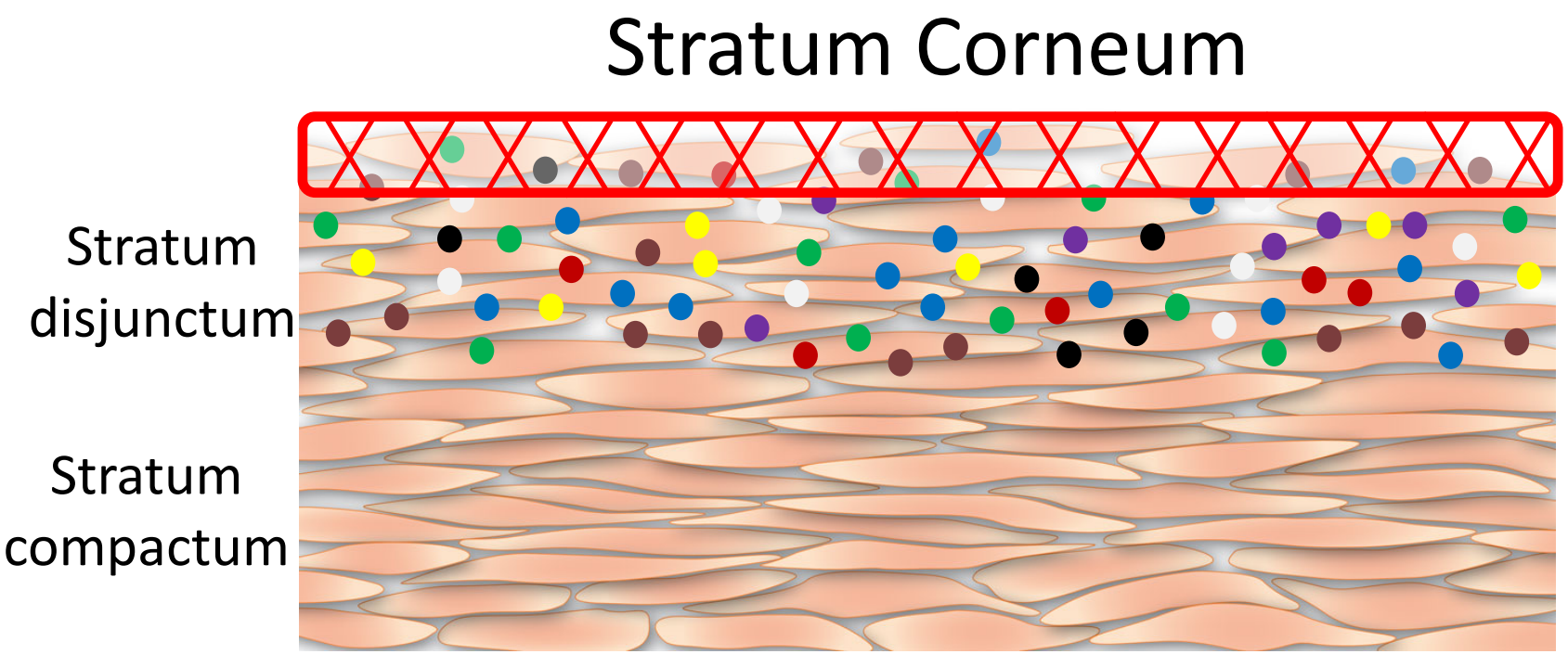
“Eigenbiome”
(= ‘own biome’)

Skin Renewal = Microbial Renewal



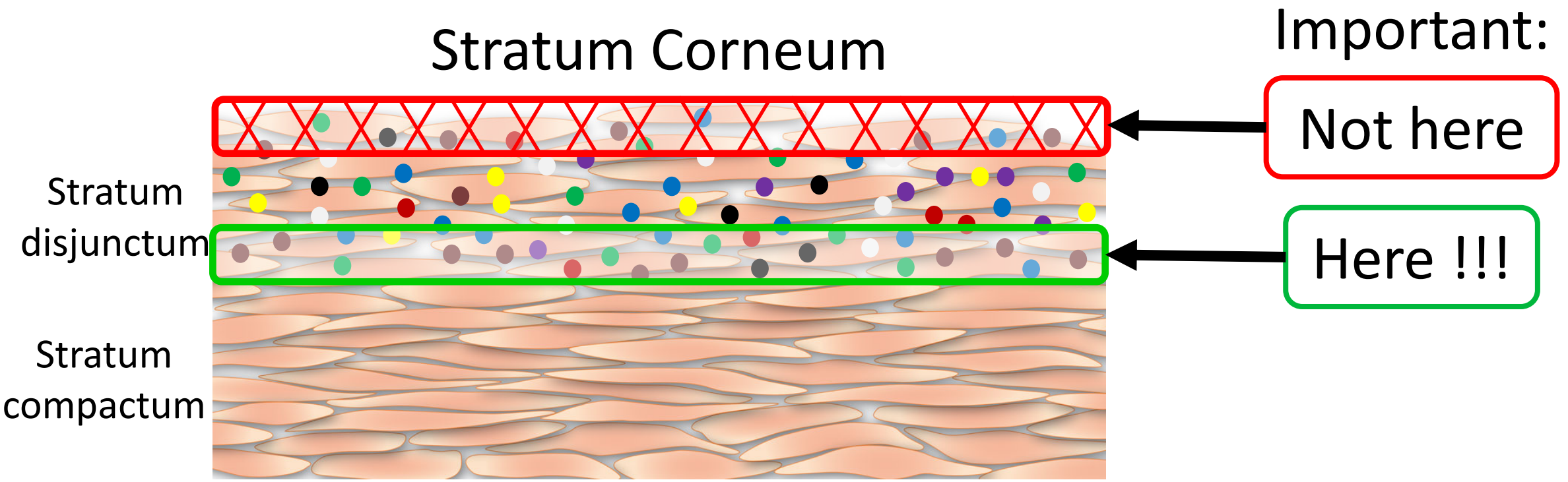
They then adhere to corneocytes and move upwards with them

Microbes at the surface of skin do not matter

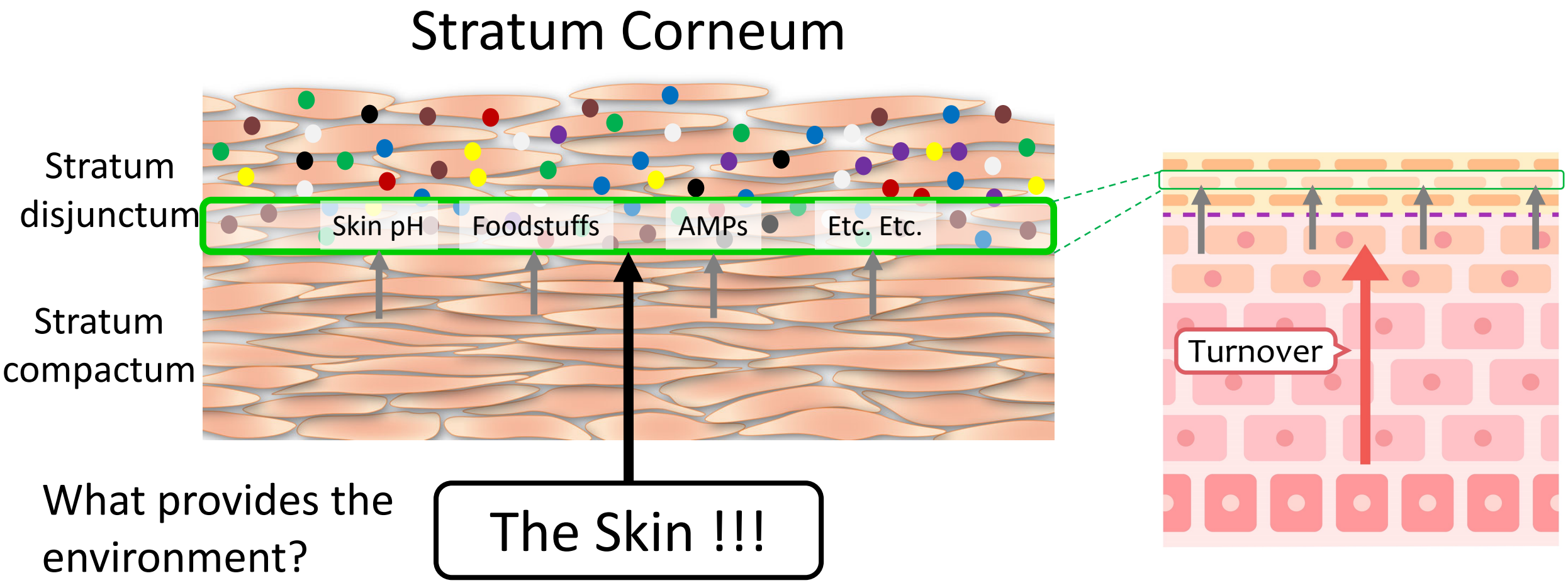


At the surface they are shed / killed / washed away / Etc.

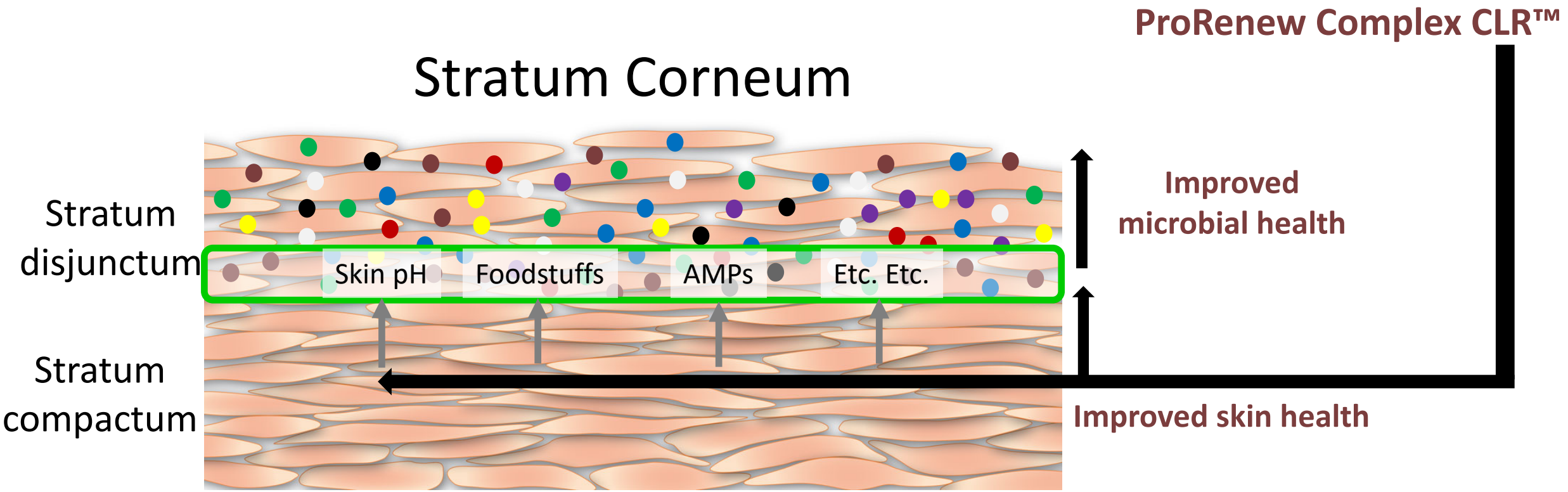
The environment inside the skin matters



Epidermal Turnover dictates the Epidermal Microbiota



Epidermal Turnover dictates the Epidermal Microbiota

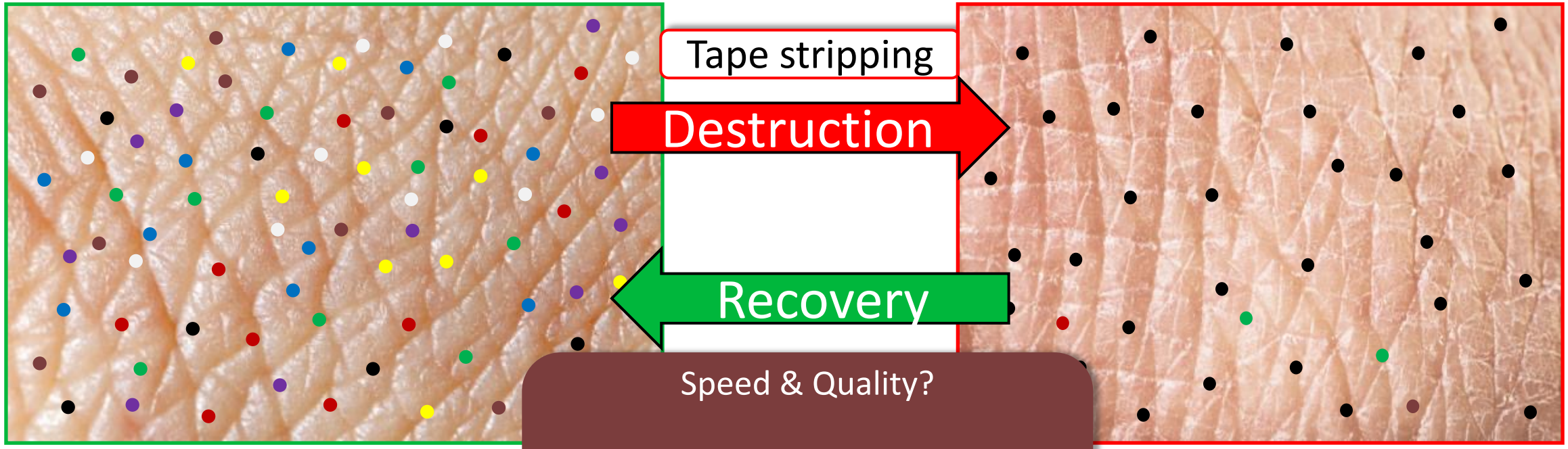


The Skin Microbiome

In vivo study

The Actual Study

Healthy Skin and Microbiome



- ✓ High richness (= many species)
- ✓ High diversity (= even distribution among species)

Speed & Quality?

Treatments:
3% ProRenew Complex CLR™
or 1% Prebiotic (FOS) or Placebo

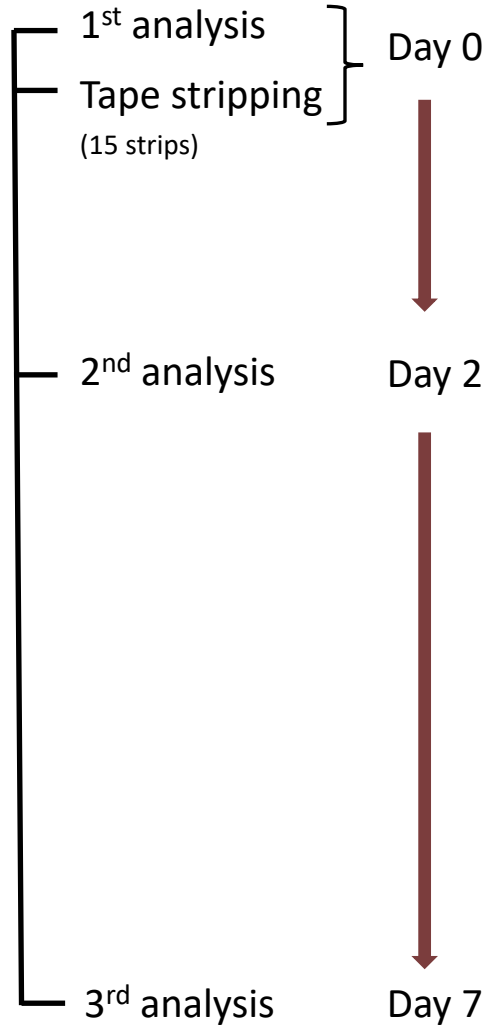
- ✓ Reduced richness
- ✓ Reduced diversity
- ✓ Disturbed Stratum Corneum

ProRenew Complex CLR™ for the skin microbiome

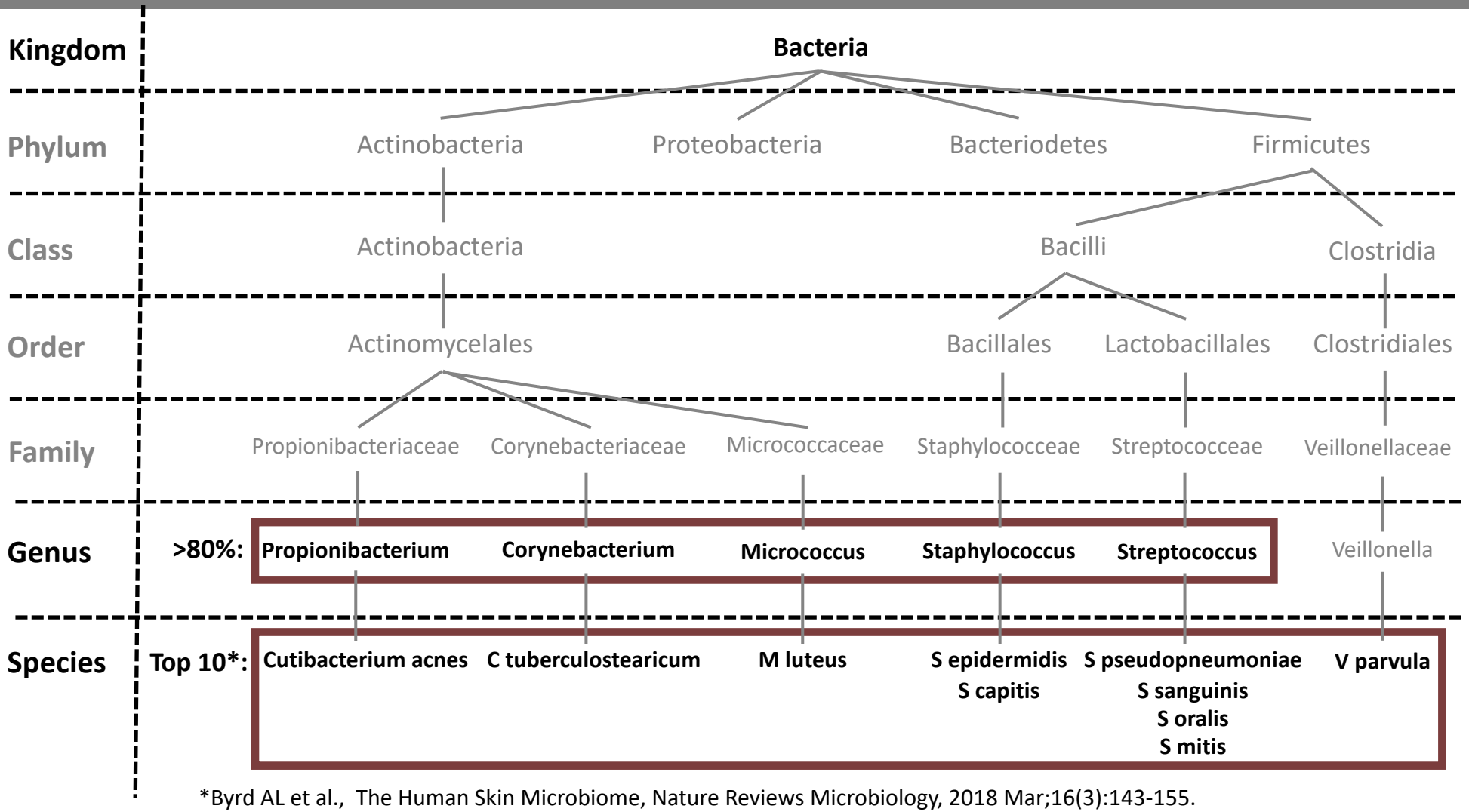
Approach:

- Analysis of microbiota (16S rRNA gene sequencing)
- Disruption of microbiota through tape stripping
- Kinetic of recovery of composition?
- 17 volunteers with normal skin

In co-operation with UNIKA-T, a research association between University Hospital Augsburg, University of Augsburg, Technical University of Munich (TUM), and the Ludwig-Maximilians-University Munich (LMU)



Analysis on multiple levels of bacterial taxonomy

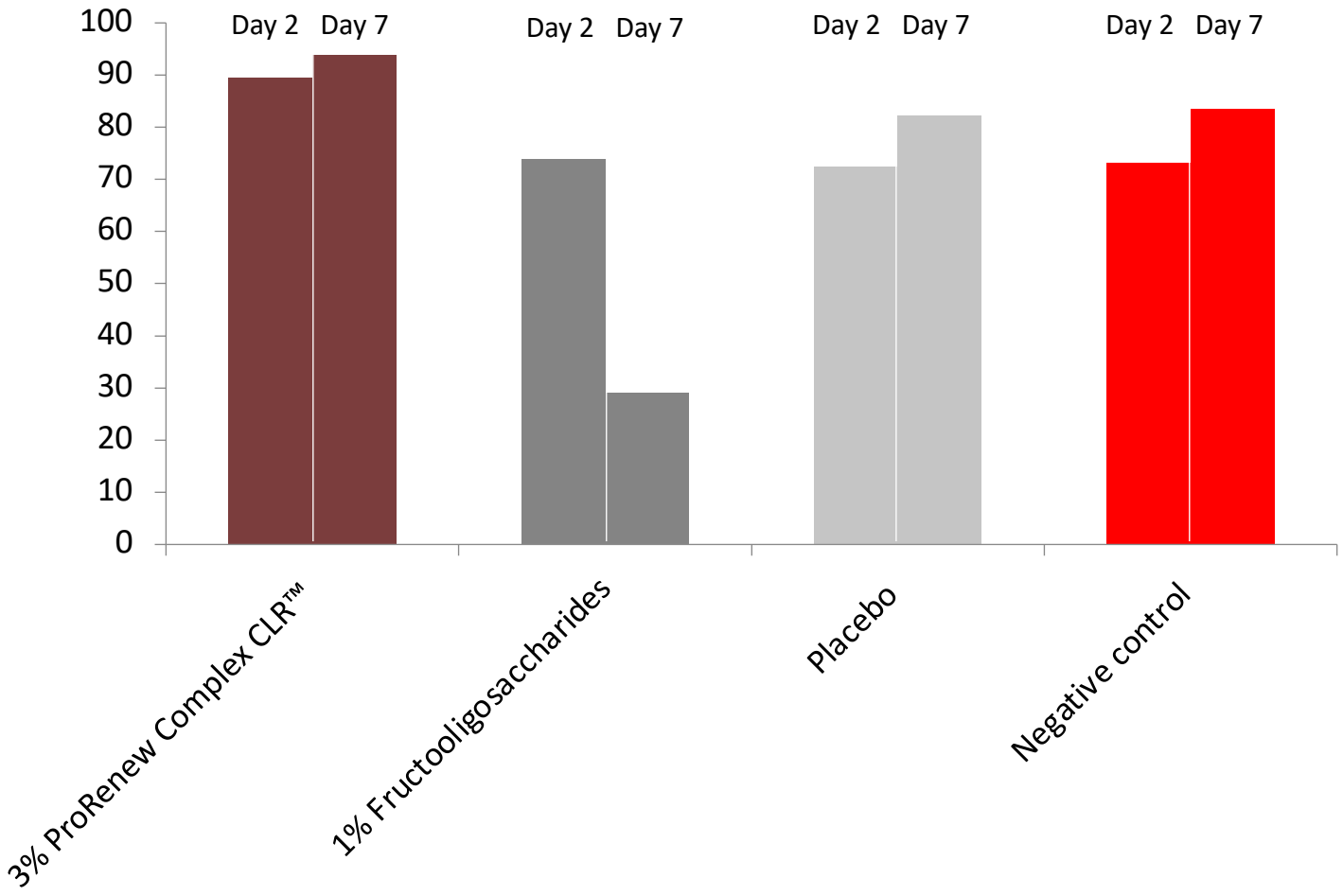


*Byrd AL et al., The Human Skin Microbiome, Nature Reviews Microbiology, 2018 Mar;16(3):143-155.

Distribution of 5 main genera

Similarity with positive control (%)

Calculated from results of different treatments on 5 main genera (Propionibacterium, Staphylococcus, Micrococcus, Corynebacterium, Streptococcus), weighted for relative abundance of the genera (positive control set at 100%).



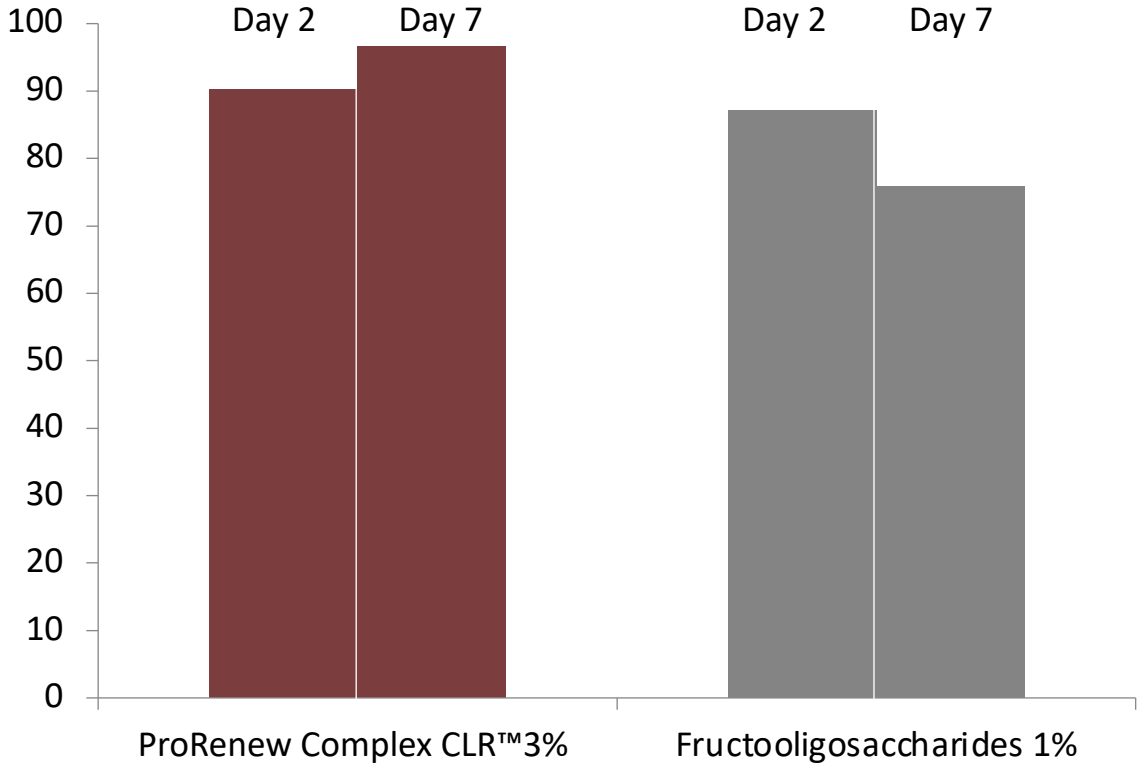
Distribution of 10 main species

Top 10 bacteria on inner forearm:

Micrococcus luteus
Cutibacterium acnes
Staphylococcus capitis
Staphylococcus epidermidis
Streptococcus pseudopneumoniae
Streptococcus sanguinis
Streptococcus oralis
Veillonella parvula
Corynebacterium tuberculostearicum
Streptococcus mitis

Byrd AL et al., The Human Skin Microbiome, Nature Reviews Microbiology, 2018 Mar;16(3):143-155.

Top 10 species abundance, similarity with positive control (untreated, undamaged, set at 100%):



ProRenew Complex CLR™

Works on:

- Skin Renewal (Speed and Quality)
- Microbiome Recovery

Application:

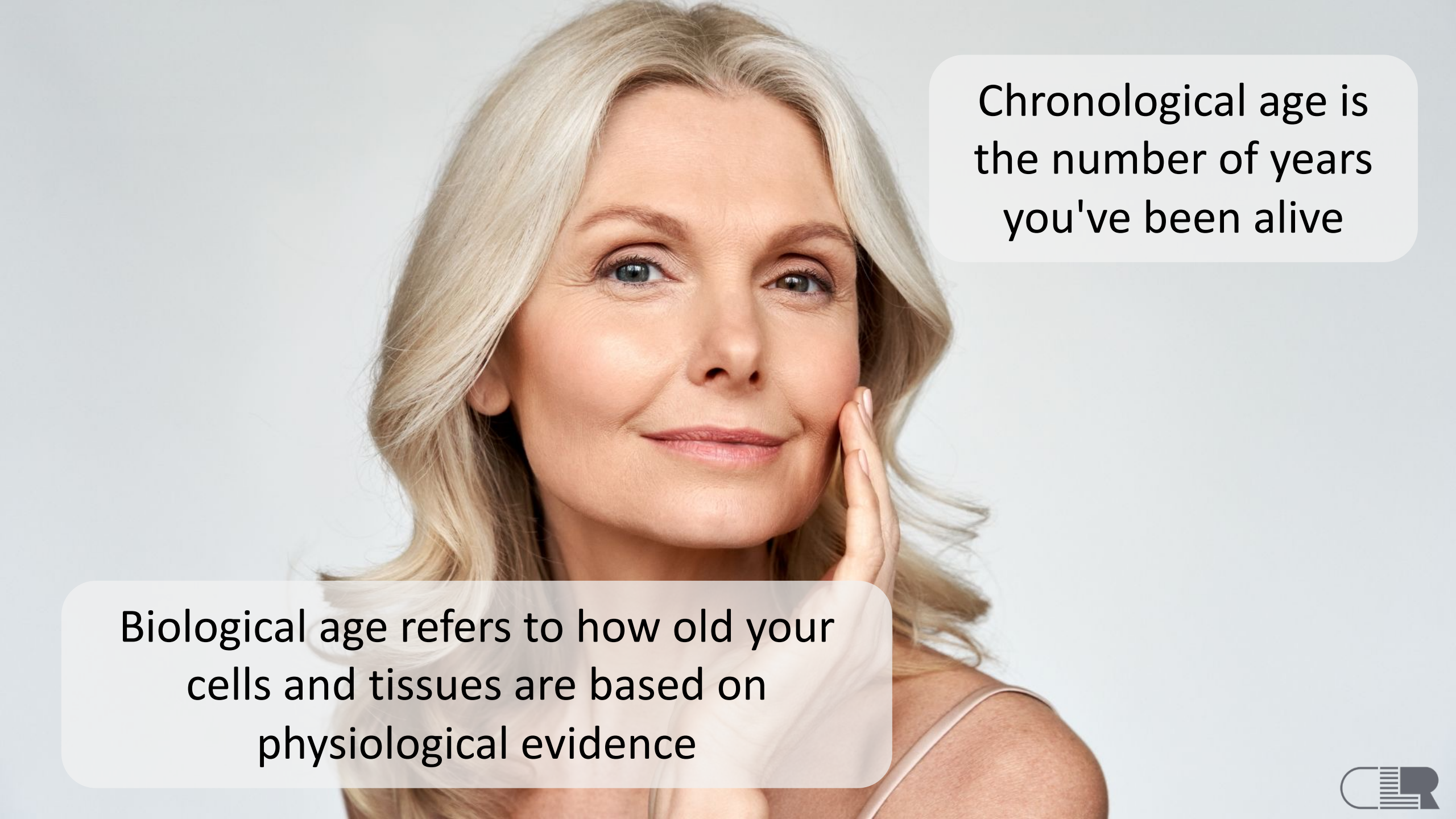
- “Historically” for Skin - anti-age
- Shampoo formulations (New studies short time: 1 week, 3 washes)
- Intimate care (in addition, works at low pH and come from Lactococus Lactis)

G+C Complex CLR™

The BioGenetiC Codex
Skin cell Synchronization & Repair

People age at different rates and in different qualities

Chronological age and biological age are not the same

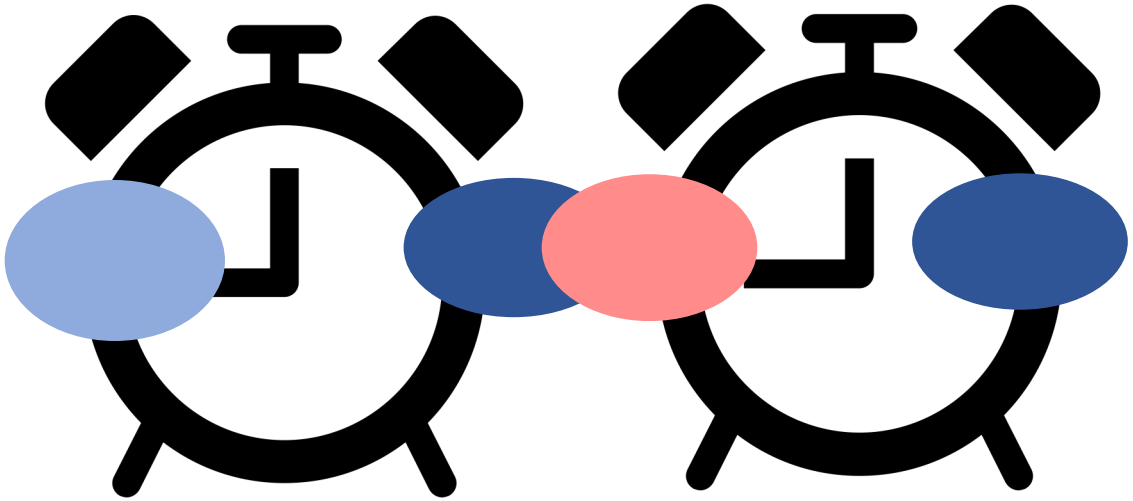


Chronological age is the number of years you've been alive

Biological age refers to how old your cells and tissues are based on physiological evidence

Lifestyle factors – Skin aging is not just genetic

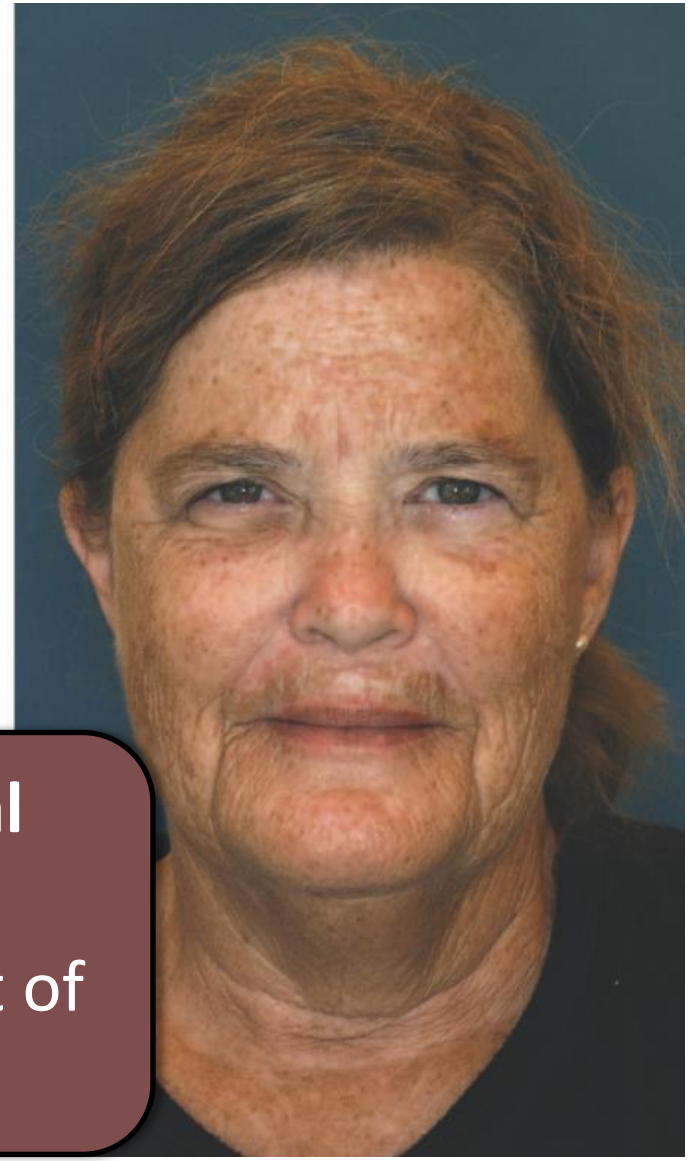
Twins. Age: 61 years old. Difference: approx. 10 hours of sun exposure per week



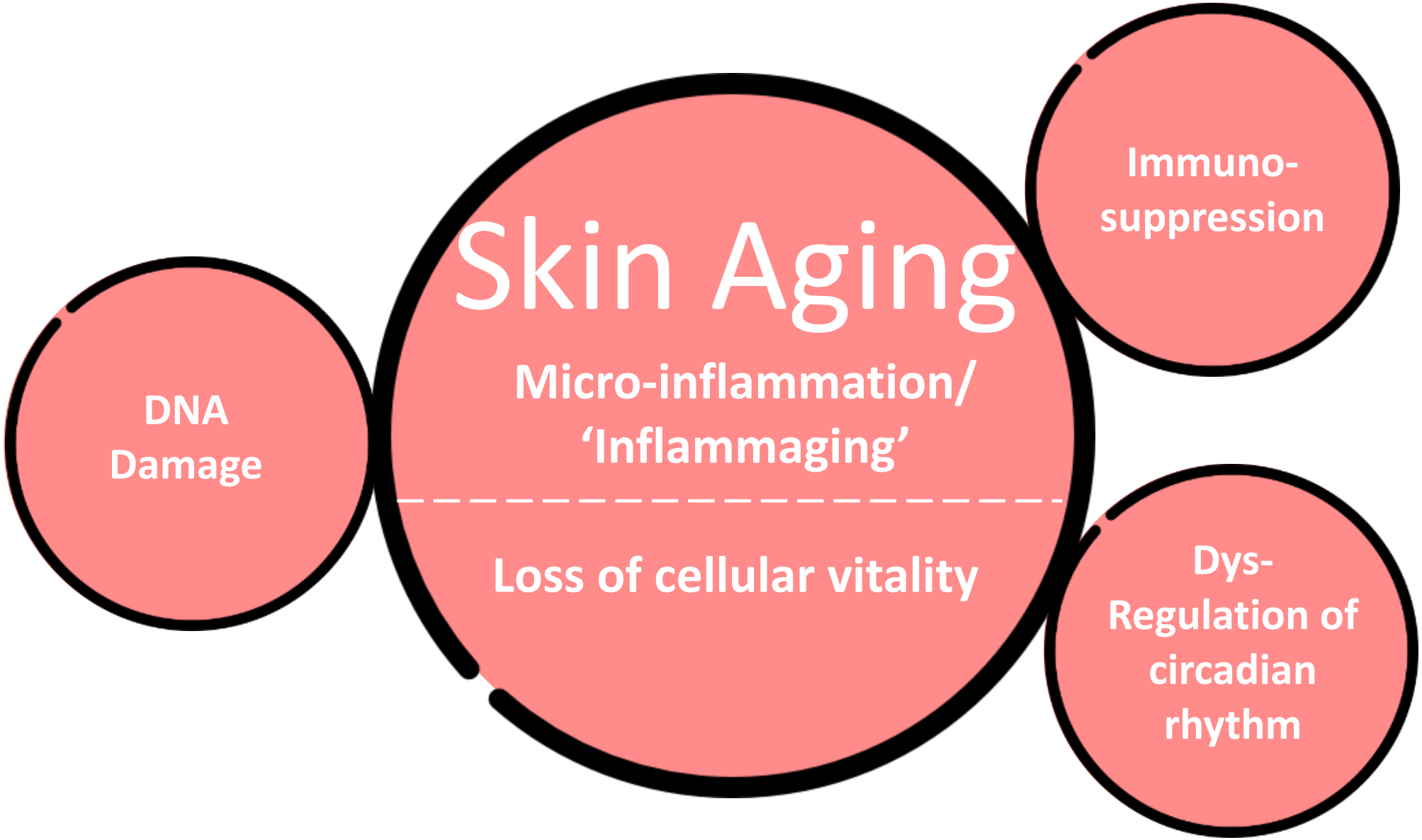
Chronological aging: Synchronized skin

Photographs from: Plast Reconstr Surg. 2009 Apr; 123(4):1321-1331. doi: 10.1097/PRS.0b013e31819c4d42. Factors contributing to the facial aging of identical twins Bahman Guyuron et al.

Biological aging: Skin, 'out of sync'



The vicious circles of Skin Photoaging



G+C Complex CLR™: potentiate DNA repair

Accumulation of DNA damage in skin cells is a key event in skin aging

The Biogenetic Codex: a Cycle of DNA damage and repair

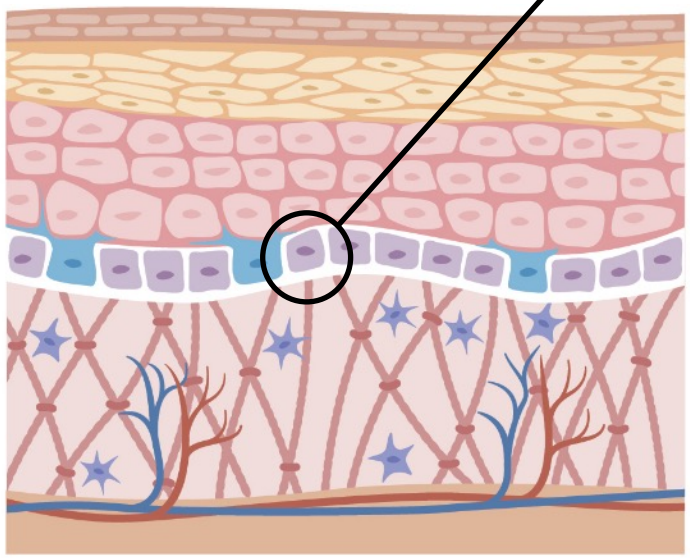
- **DNA damage is constant**: A single skin cell suffers multiple DNA mutations per day, mainly as a result of UV light

- **DNA repair is constant**: DNA is not immune to damage, and therefore it is vital for DNA to repair itself in order to maintain normal cell function

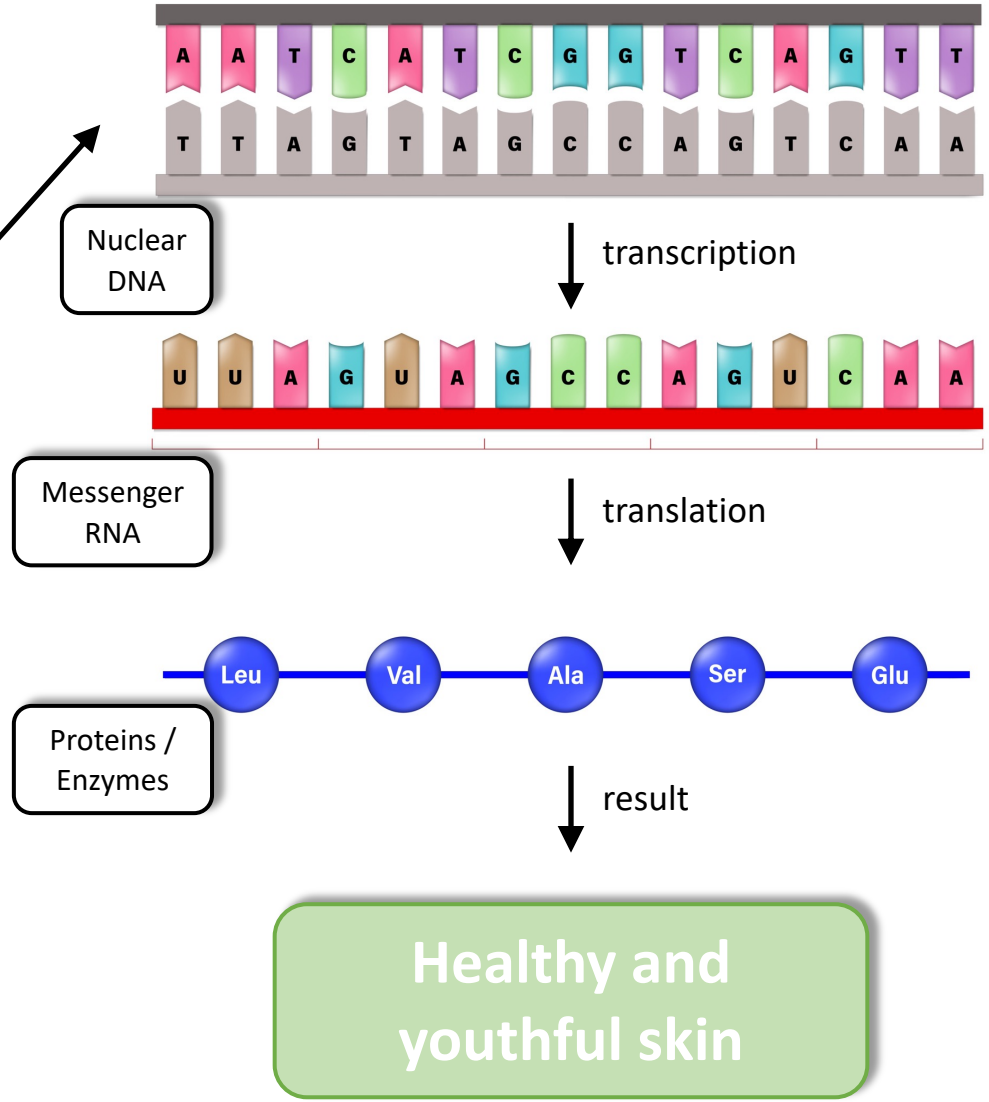
- Numerous response mechanisms to DNA damage have been developed by cells to specifically recognize and repair each type of damage

G+C Complex CLR™

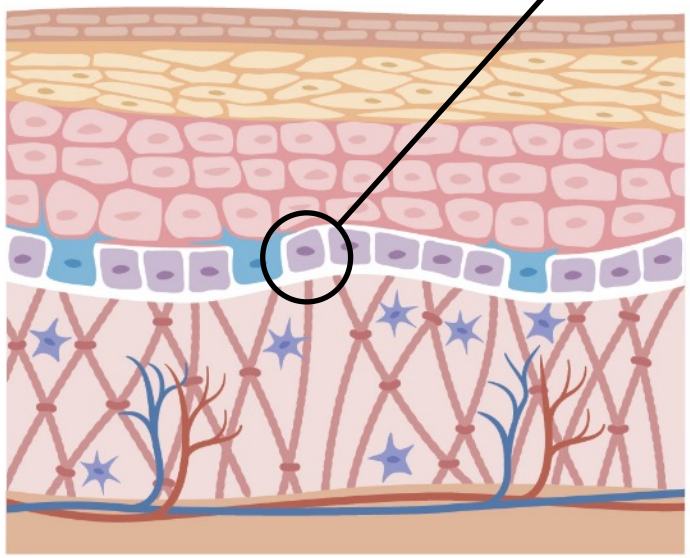
Skin cells youthful functionality depends DNA integrity

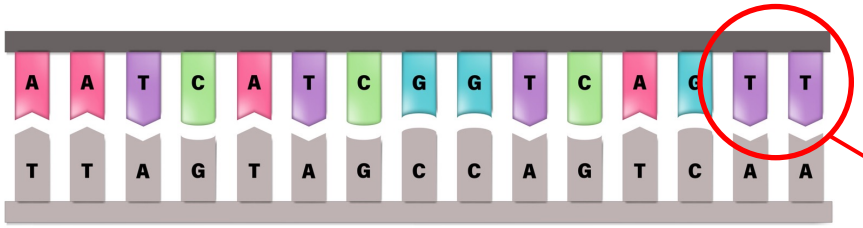


G+C Complex CLR™

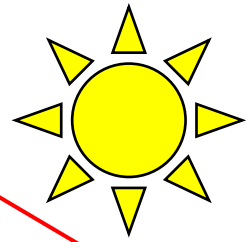


Potential for CPD mutations everywhere in the DNA





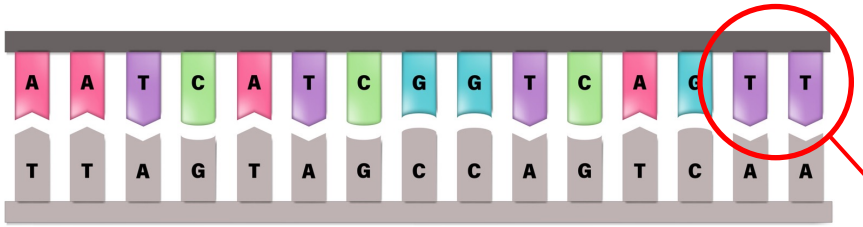
Two Thymine (T) bases with pyrimidine groups next to each other

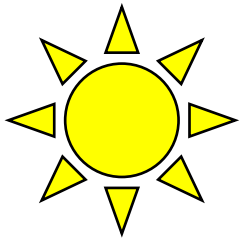


Potential for DNA mutation: CPD (Cyclobutane Pyrimidine Dimer), i.e. Thymine Dimer

G+C Complex CLR™

Skin cells youthful functionality depends DNA integrity

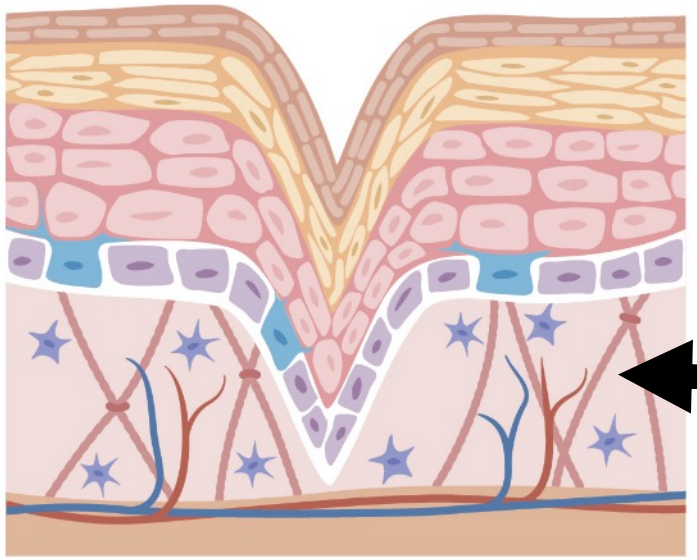




Potential for CPD mutation

Disruption of normal cellular functionality and viability, Inflammaging

Skin Photoaging



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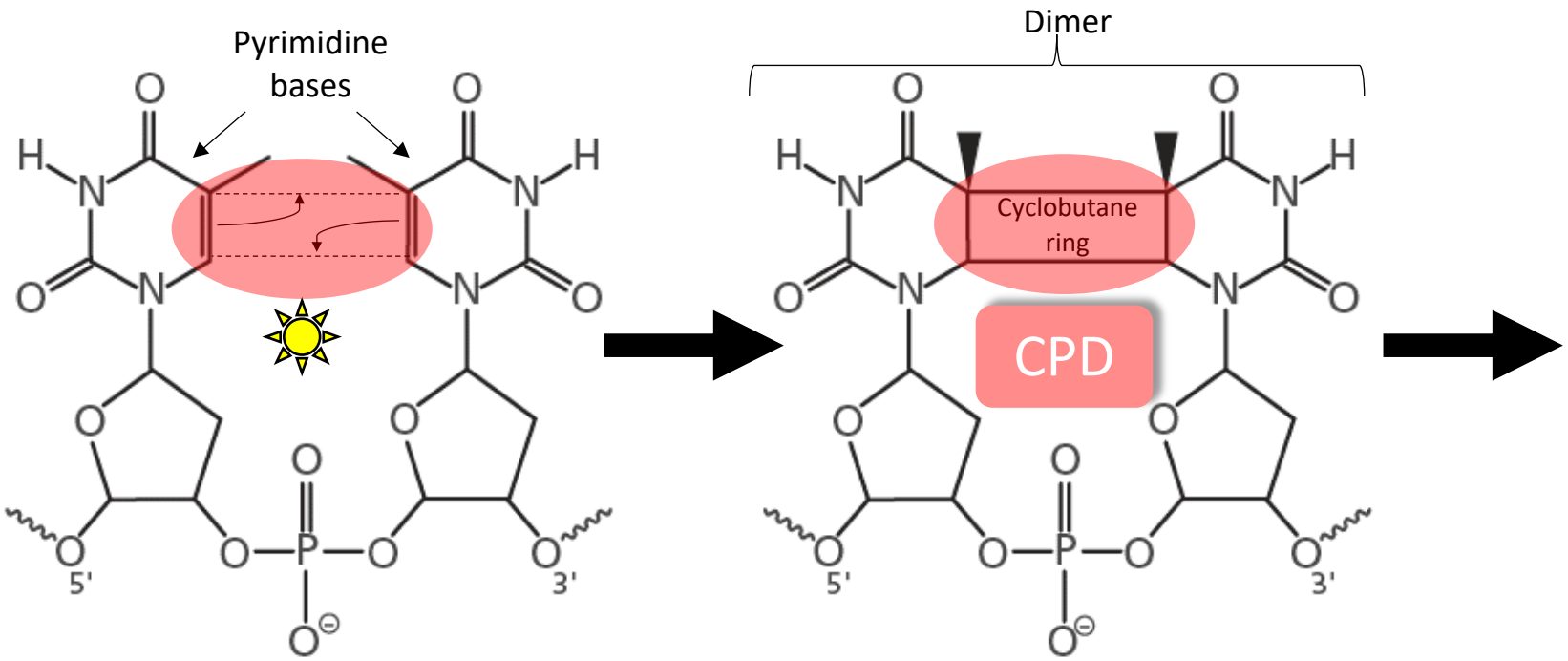
CPD's: Cyclobutane Pyrimidine Dimers

Origin of CPD's:

Sunlight induces a photochemical reaction leading to a cyclobutane ring between two adjacent pyrimidine bases



DNA distortions:

- Affecting important cellular processes such as DNA replication and transcription
- Compromising cellular viability and functional integrity

G+C Complex CLR™

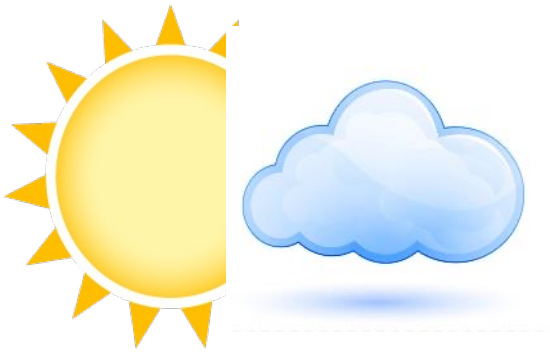
G+C Complex CLR™: stabilizes skin's circadian rhythm

When the chronobiology of skin is desynchronized, DNA repair is disturbed

Immuno-suppression

Dys-Regulation of circadian rhythm

Skin´s natural biorhythm dictates the DNA damage/repair cycle

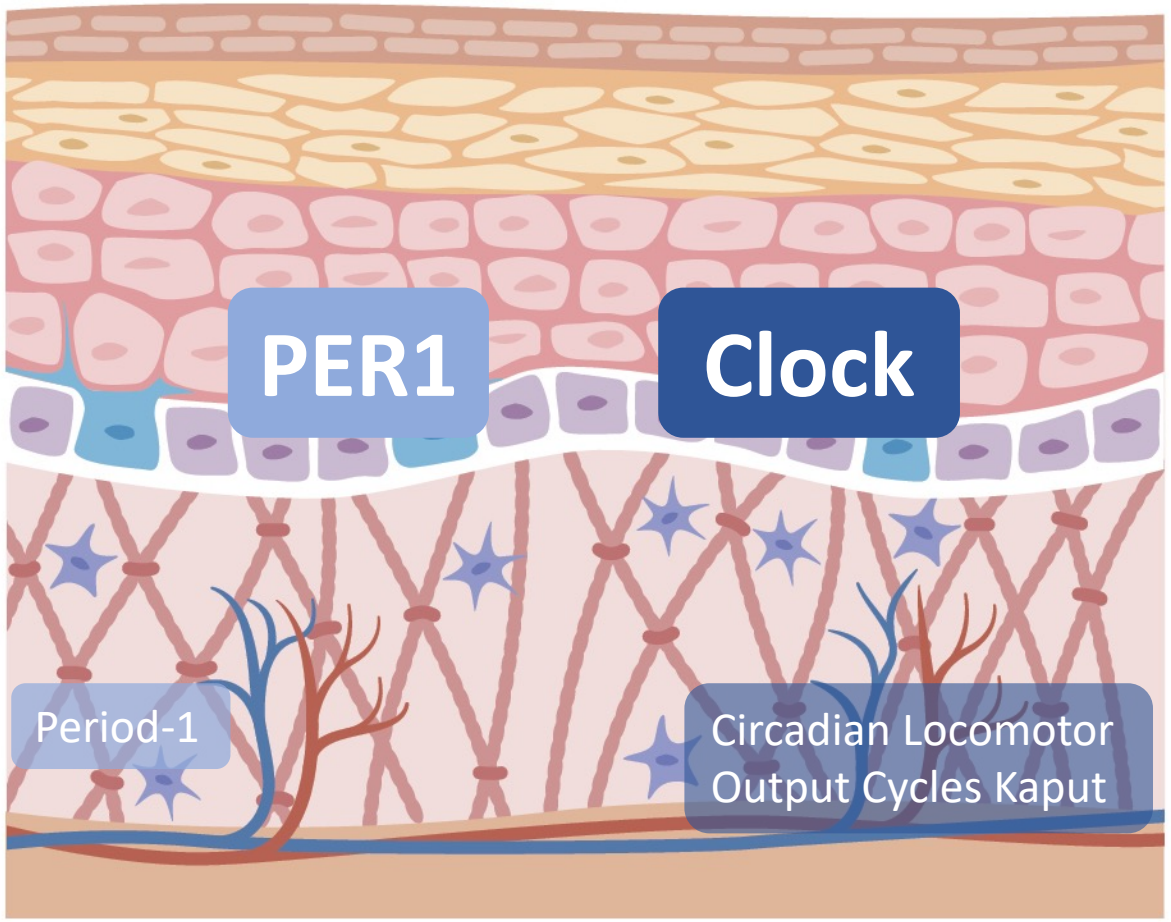


DAMAGE

REPAIR

G+C Complex CLR™

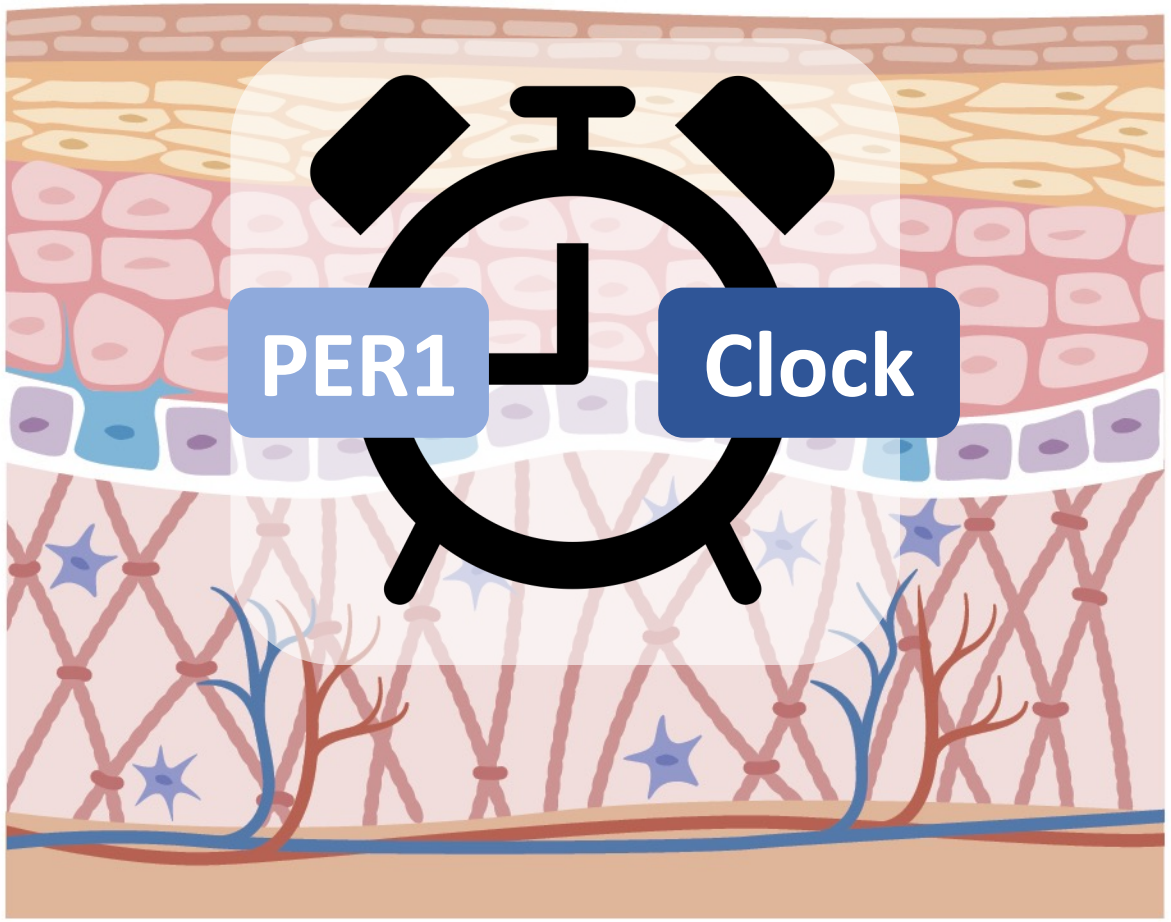
Cell biology of skin's circadian rhythm



Key Proteins in dictating the chronobiology of our skin

G+C Complex CLR™

Cell biology of skin’s circadian rhythm

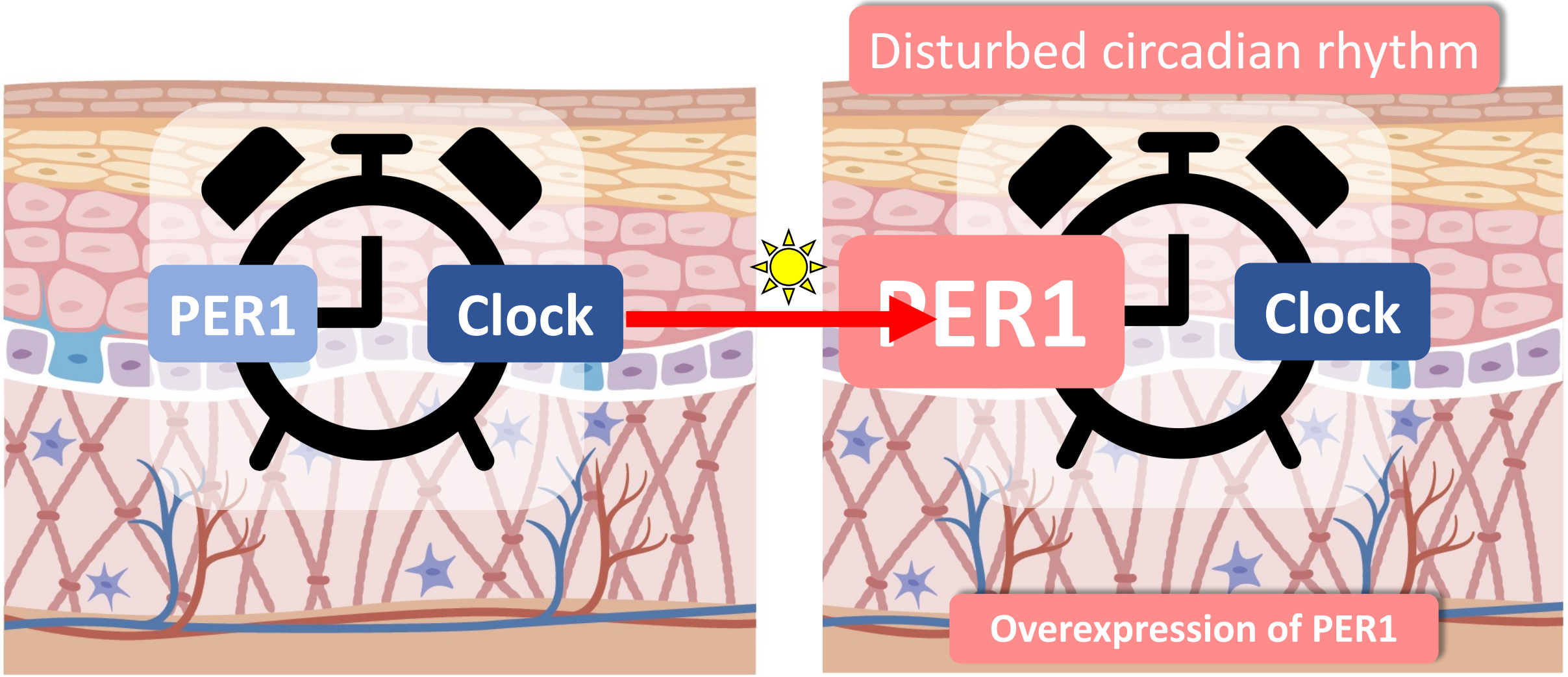


Balance between PER1 and CLOCK dictates a 24 hour-rhythm for our skin cells

Essential for DNA Repair

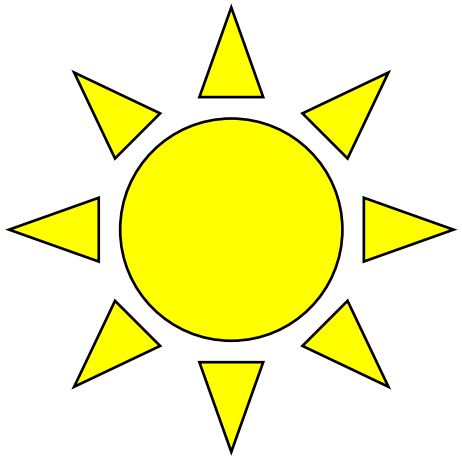
G+C Complex CLR™

Cell biology of skin’s circadian rhythm

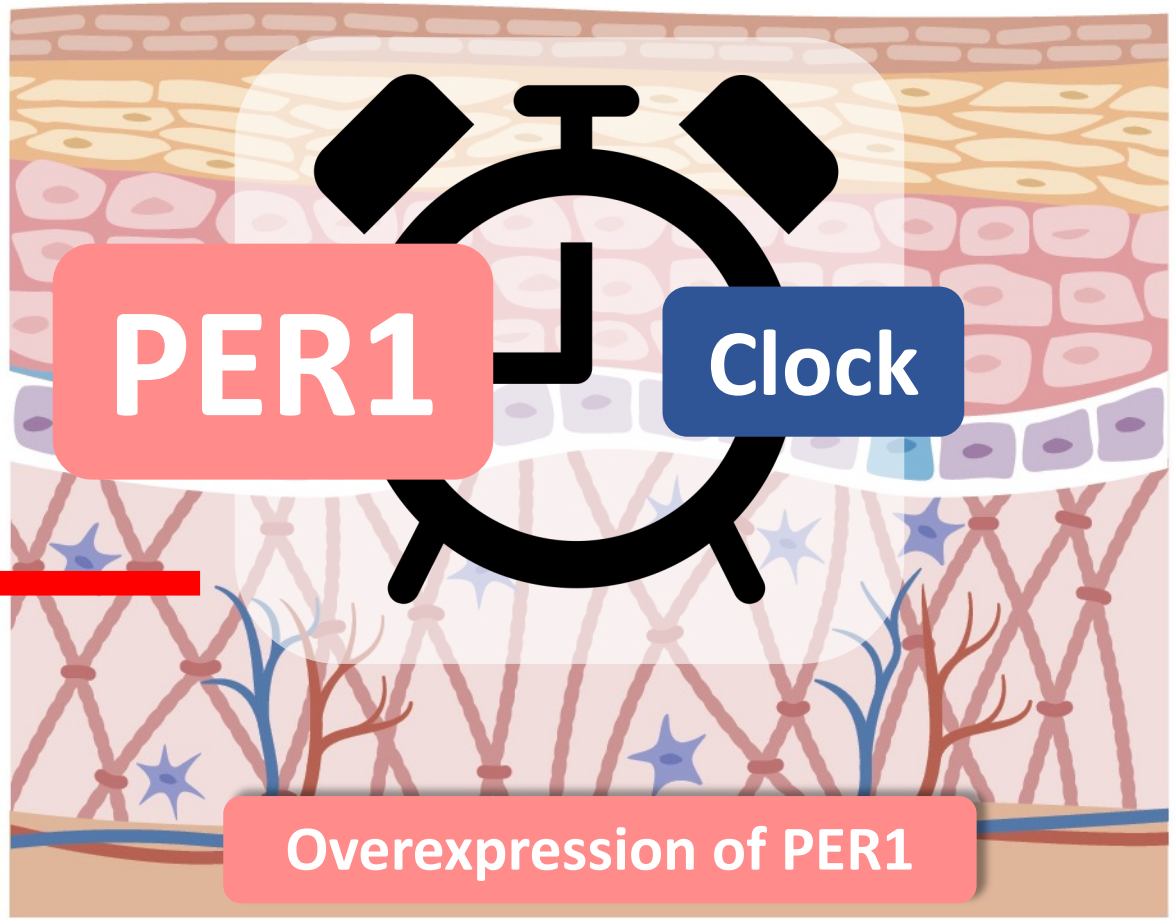


G+C Complex CLR™

Cell biology of skin’s circadian rhythm



Disturbed
DNA repair



G+C Complex CLR™

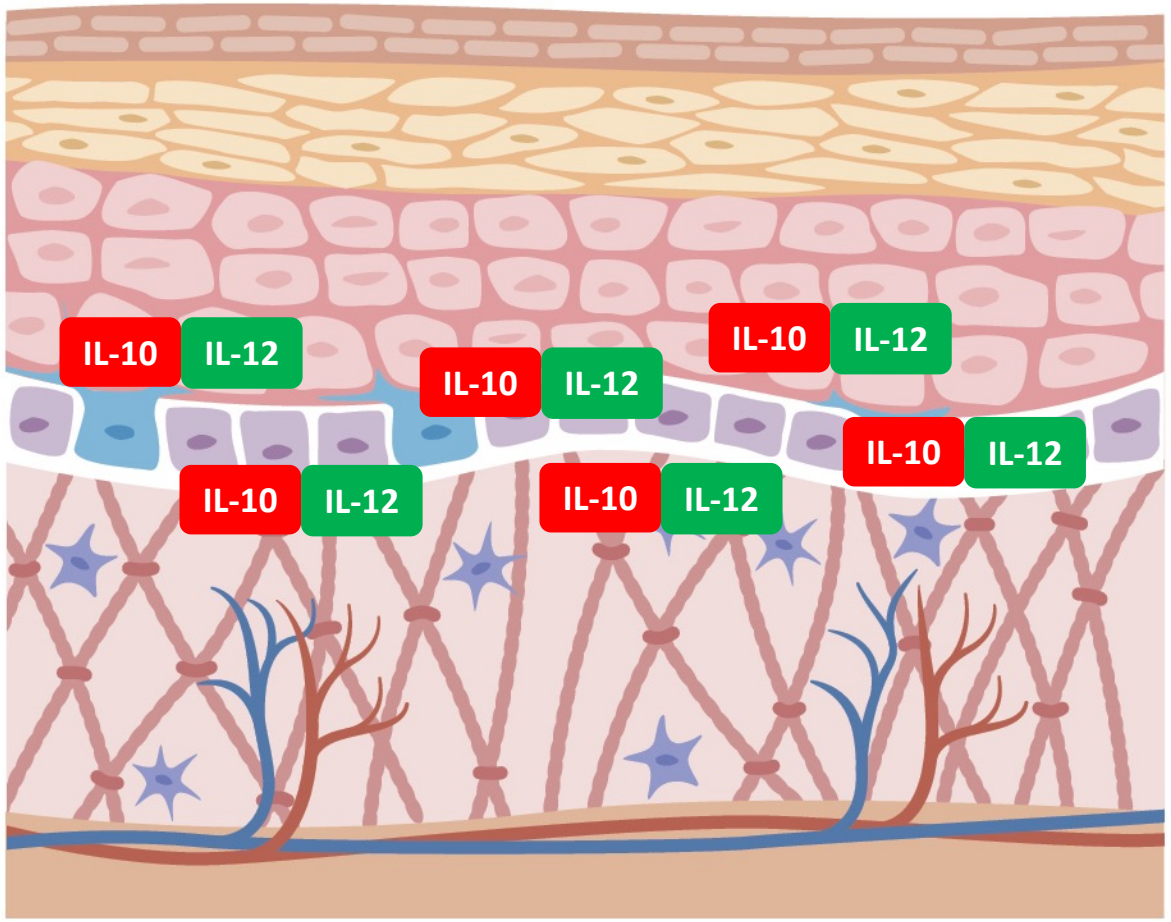
G+C Complex CLR™: immunostimulating

UV-radiation leads a weakening of the skin cells' ability to repair DNA damage

Immuno-suppression

Dys-Regulation of circadian rhythm

Skin immunology: the Ideal Situation

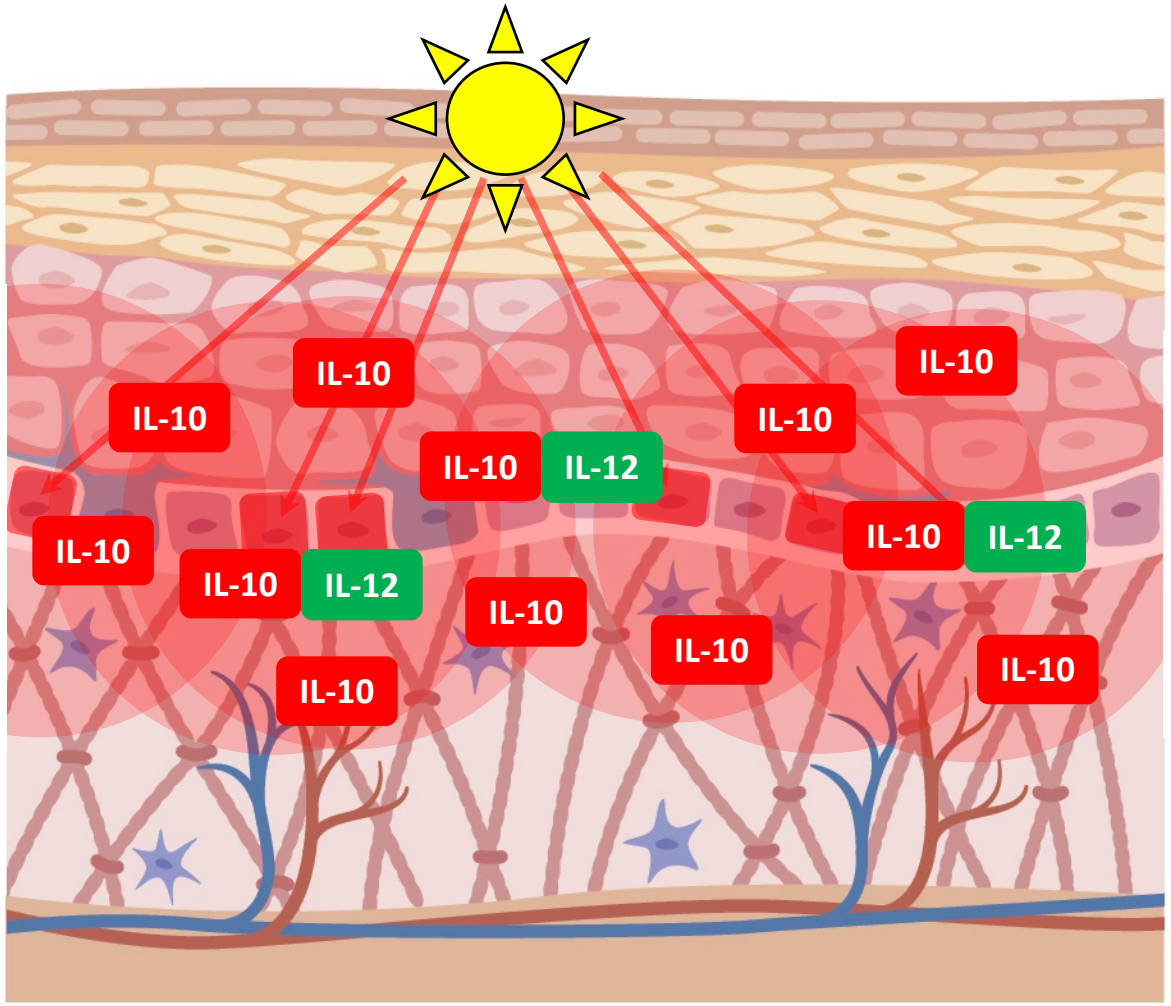


Unchallenged skin: a perfect immunological balance

- IL-10: Immunosuppressive (reduces DNA repair)
- IL-12: Immuno-stimulating (induces DNA repair)

G+C Complex CLR™

UV-induced Immunosuppression: IL-10 vs. IL-12



G+C Complex CLR™

UV-induced DNA damage induces IL-10 and reduces IL-12

↓

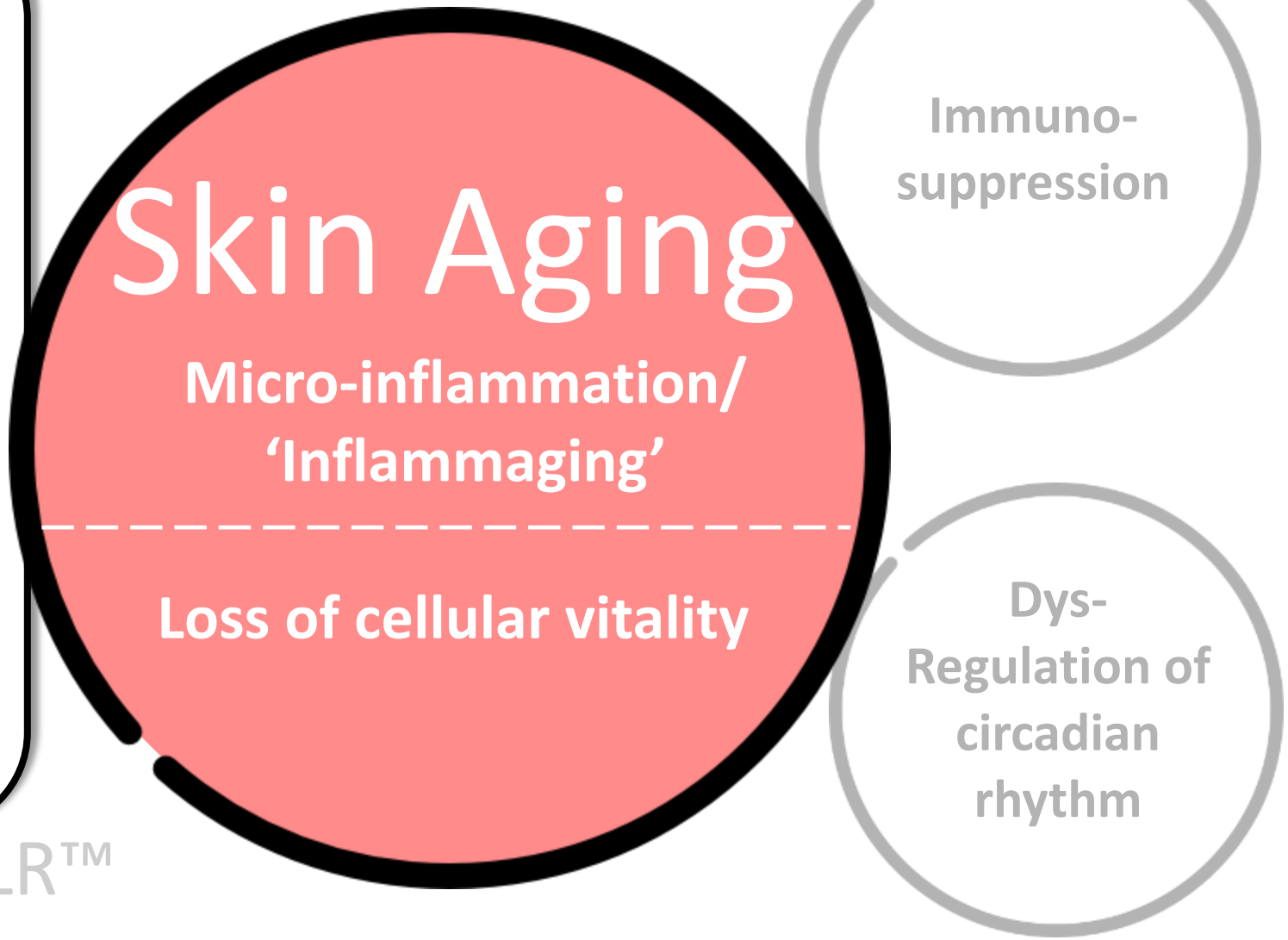
Immunosuppression

↓

Reduced ability to repair DNA

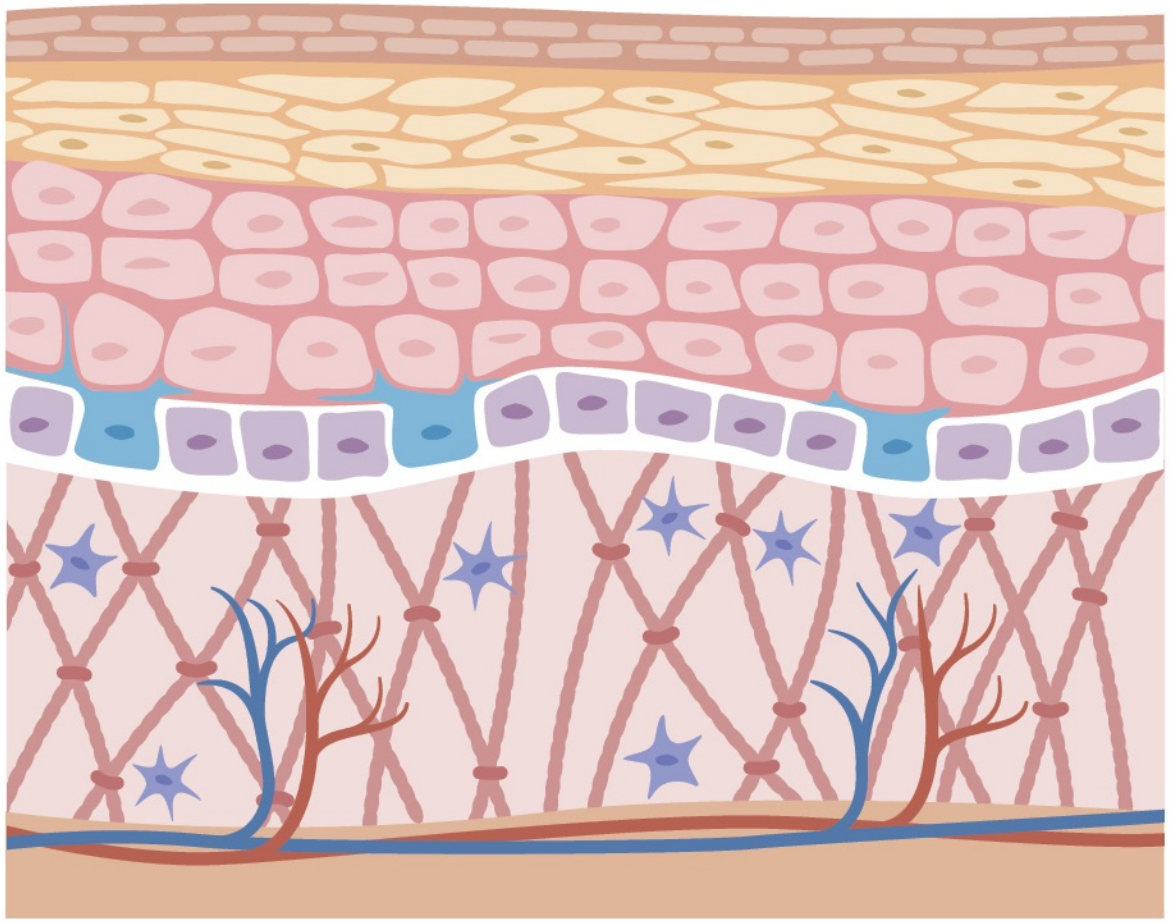
G+C Complex CLR™: acts against biological skin aging

The resulting accumulation of DNA damage plays a key role in inflammaging and loss of cellular functionality



G+C Complex CLR™

Skin ‘Inflammaging’: the Ideal Situation

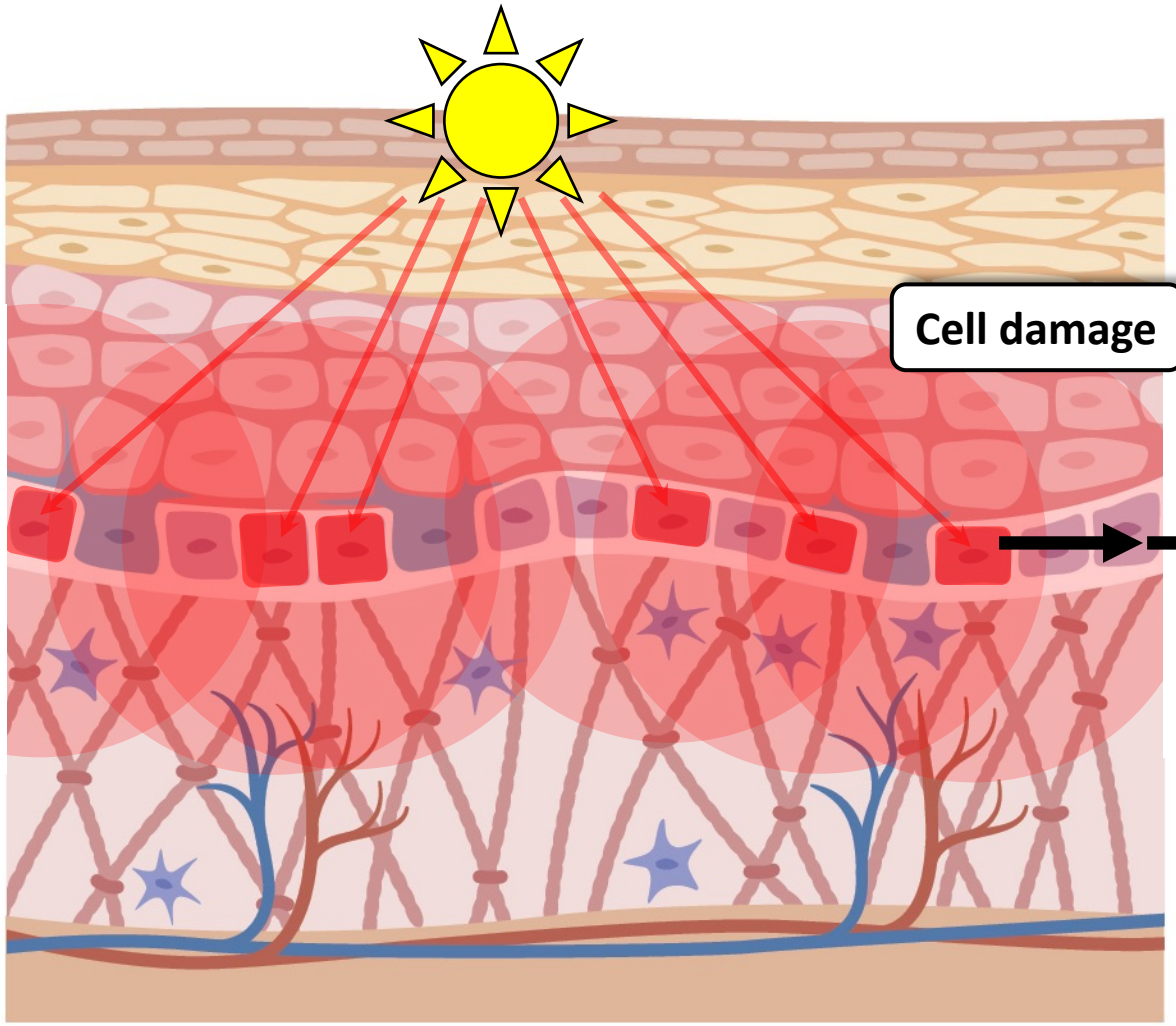


G+C Complex CLR™

Unchallenged and Youthful Skin:

- No microinflammations
- No breakdown of collagens
- ‘Inflammaging’ reduced to the minimum

UV-induced cell damage leads to ‘Inflammaging‘

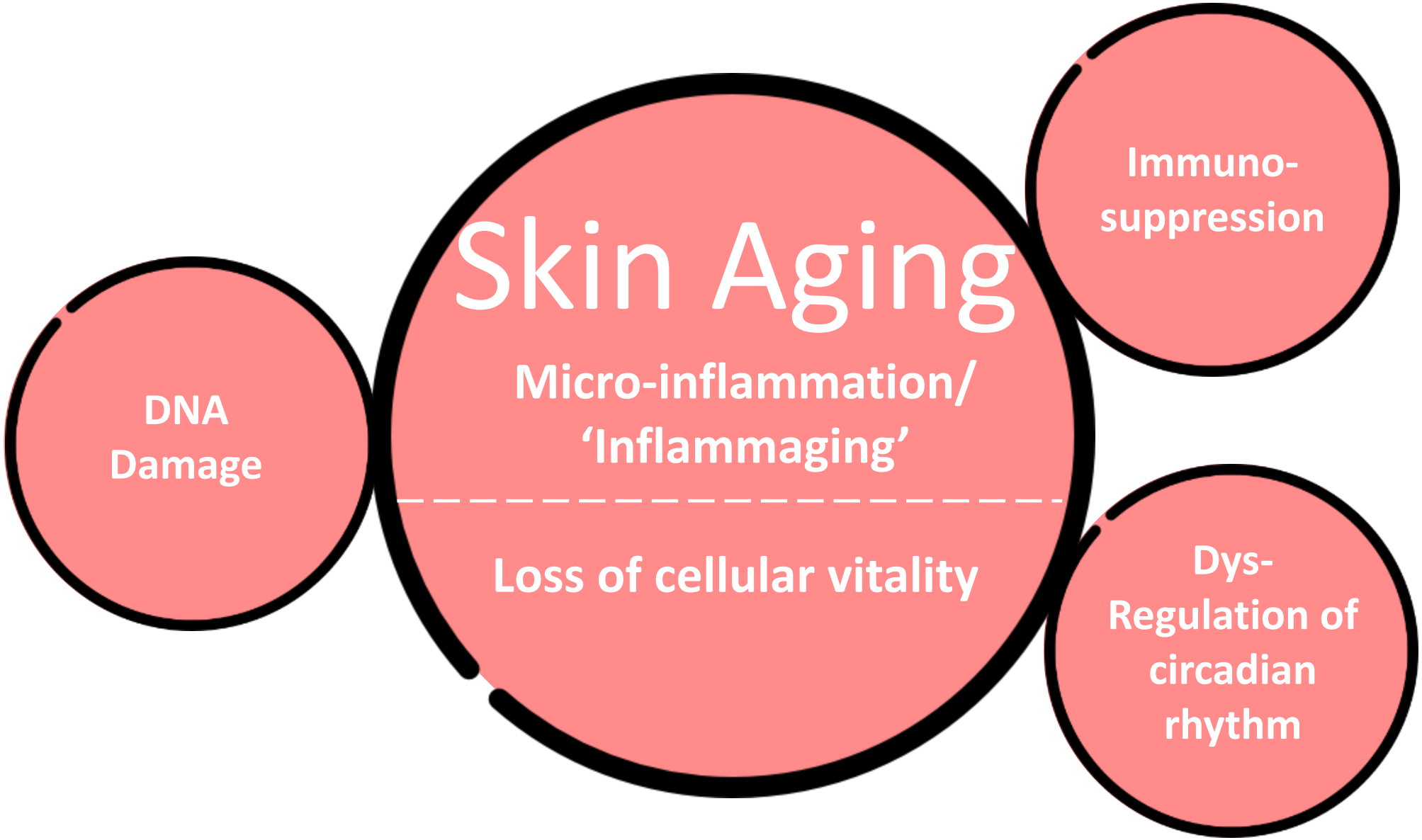


Mediators in ‘inflammaging’, e.g.:

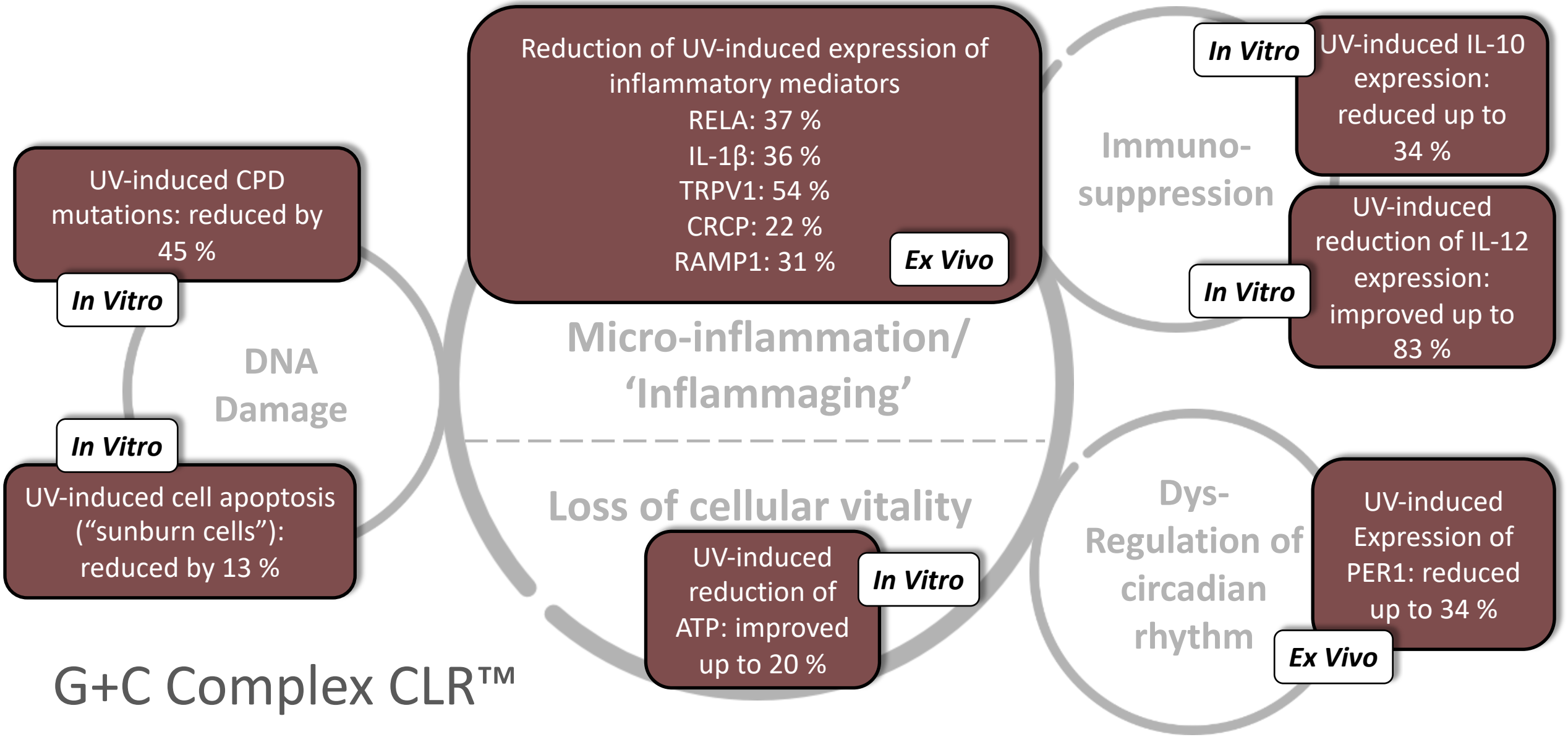
- **RELA**
- **IL-1β**
- **CRCP**
- **RAMP1**
- **TRPV1**

G+C Complex CLR™

The vicious circles of Skin Photoaging



G+C Complex CLR™: breaking the vicious circles of Photoaging



G+C Complex CLR™

G+C Complex CLR™

Works on:

- Reduction of UV-light accumulation damage
- Activates and harmonizes the skin´s circadian clock genes
- Reduces inflammatory mediators and acts against inflammaging

Application:

- Skin - Anti-age
- All formulations: Night and Day cream
- Prevention/reduction of Photoaging

Obrigado!

FRANCISCO.CLR_BERLIN

fernandez@clr-berlin.com