

$\beta$ -Defensin 2 on RHE

## Targeting inflammasome mechanisms: a possible role of the microbiota in skin ageing

Marisa Meloni, Roberto Ferrari, Francesco Carriero

# SKIN AGEING and MICROBIOTA ...?

<p>International Journal of Cosmetic Science</p> 	<p>Journal of Applied Microbiology</p> 
<p>Original Article</p> <p><b>The influence of age, gender and race/ethnicity on the composition of the human axillary microbiome</b></p> <p>M. Li, A. E. Budding, M. van der Lugt-Degen, L. Du-Thumm, M. Vandeven, A. Fan</p> <p>First published: 13 June 2019   <a href="https://doi.org/10.1111/ics.12549">https://doi.org/10.1111/ics.12549</a>   Citations: 2</p>	<p>Original Article</p> <p><b>Shift in skin microbiota of Western European women across aging</b></p> <p>R. Jugé, P. Rouaud-Tinguely, J. Breugnot, K. Servaes, C. Grimaldi, M.-P. Roth, H. Coppin, B. Closs</p> <p>First published: 23 May 2018   <a href="https://doi.org/10.1111/jam.13929">https://doi.org/10.1111/jam.13929</a>   Citations: 9</p>

## SKIN APPEARANCE and AGEING'S VISIBLE SIGNS

depend on the biology of many cell types and the structure – architecture of:

- I. Epidermis, Stratum corneum
- II. Dermis ECM and vascular network
- III. Hair follicle and dermopapilla
- IV. Muscles
- V. Adipose tissue

Anti-inflammatory defence and innate immunity in healthy skin: early mechanisms that can be investigated by **pre-clinical models**

# Host-Microbiota interaction: a Biology Dogma?

**Life on our planet has been possible thanks to Plants and CO<sub>2</sub> reduction.**

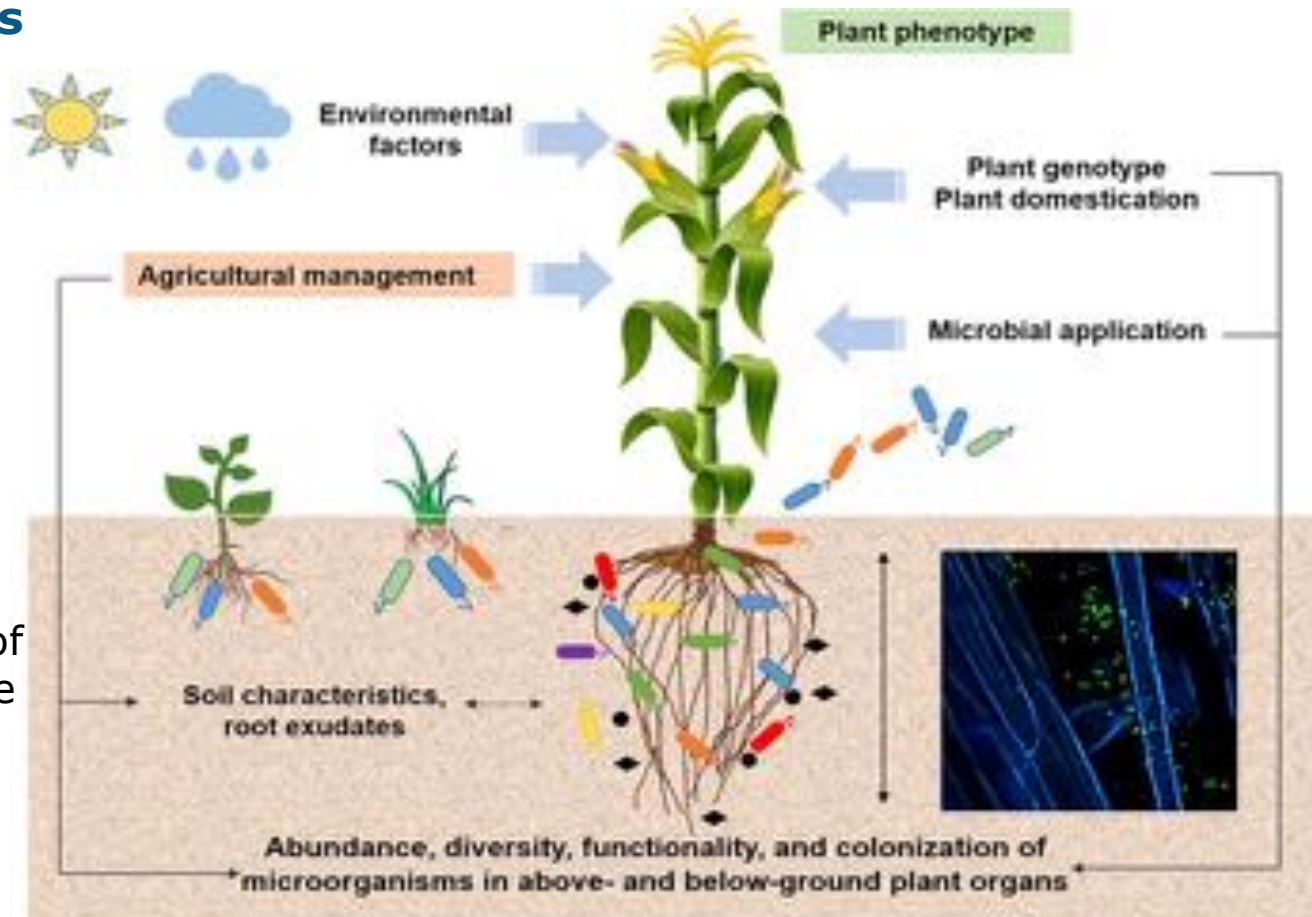
In the Earth a plethora of different microorganisms was living:

- Fungi
- Bacteria
- Archea

They have colonized both the root apparatus and above-the-ground organs.

They are localized on the surfaces of plants or they can also be inside the cells

They play an active role in enhancing plant growth, health and innate immunity.



Compant et. Al, 2019. Journal of Advanced Research, 19: 29-37

**The complex community represented by host and associated microbiota is still mostly not fully understood.**

# HUMAN MICROBIOTA: OVERVIEW

For each human cell in our body it is possible to count approximately:

3,3 bacterial cells

33,3 viral sequences

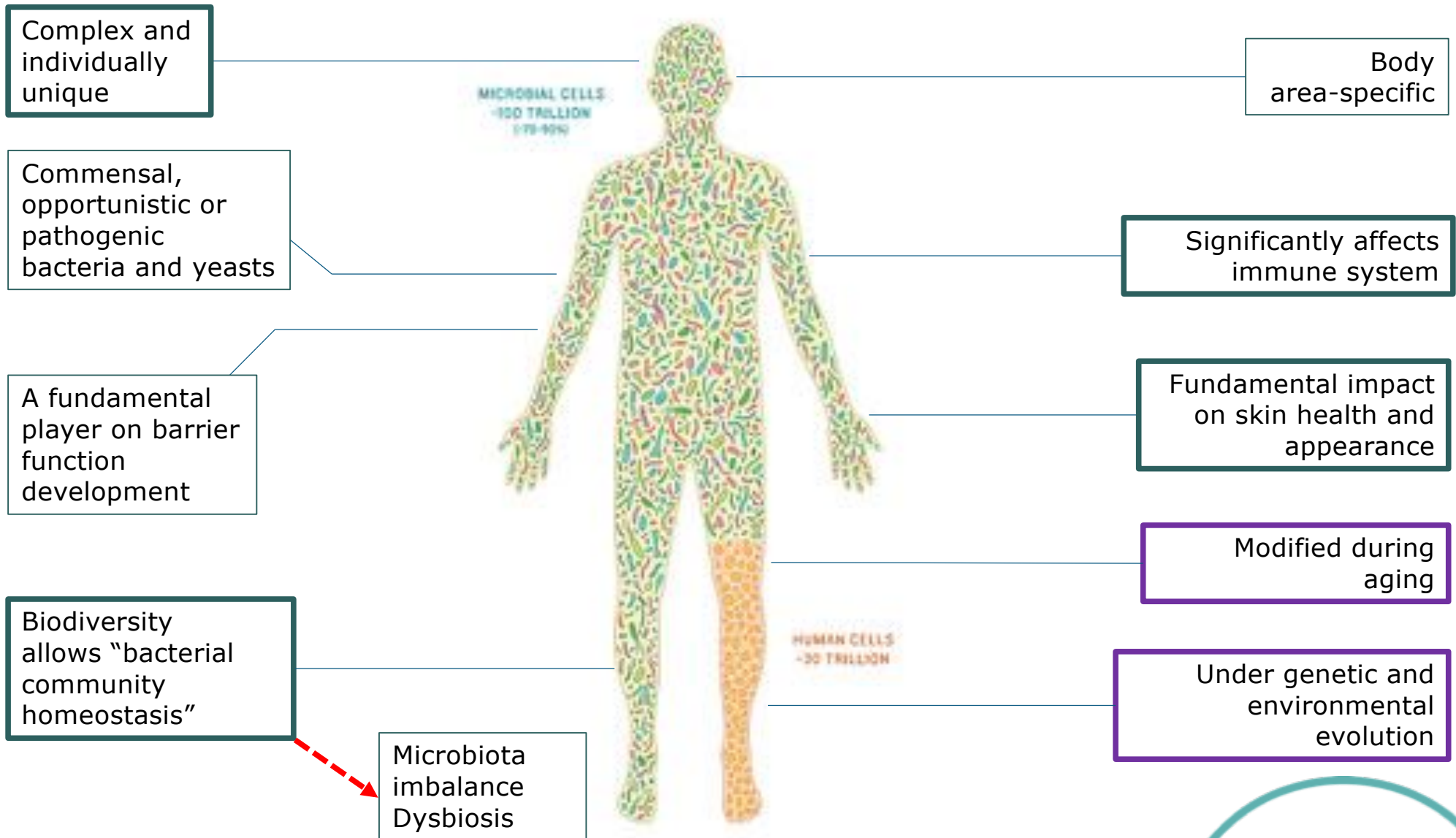
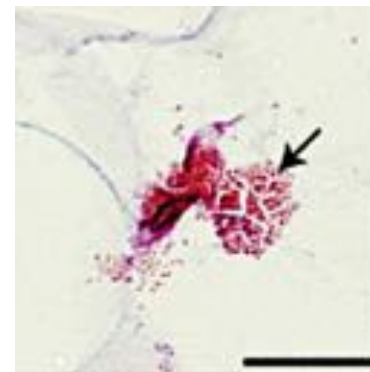


Image by: Gaby D'Allesandro / © AMNH

# Microbiota does not act only locally

Thanks to sequencing techniques the more and more complex composition of each body niches microbiota is becoming more visible ... however it doesn't allow to fully understand its role towards **host health status: biodiversity and individual uniqueness.**



Gram staining of frozen sections from **adipose tissue** of normal human facial skin (*Staphylococcus*, *Pseudomonas*). Nakatsuji *et al*, 2013. (Bar=20µm)

# Each individual microbiome is built up in a “holistic environment”

- Host behaviors impact microbiota
- Host Genetic (human races)
- Host immune system
- Diet
- Environment (UV, pollution)
- Hygiene behavior and skin physiology



# WHAT TRIGGERS SKIN AGING PROCESS?

- *Host behaviors*
- *Host Genetic (human races)*
- *Host immune system*
- *Diet*
- *Environment (UV, pollution)*
- *Hygiene behavior and skin physiology*

...the same genetic and intrinsic factors that influence skin **microbiome** have a significant impact on **skin ageing**



## Air Pollution and Skin Aging

[Tamara Schikowski](#) & [Anke Hüls](#) ✉

[Current Environmental Health Reports](#) 7, 58–64(2020)

# SKIN AGEING IS UNDER GENETIC AND ENVIRONMENTAL INFLUENCE



- Barrier function still in development
- Lower pigmentation
- High water content and plasticity

under construction



- Optimal barrier function
- Relatively homogeneous pigmentation
- Limited water loss
- Regular renewal

complex and individually unique



- Thinner epidermis
- SC increased thickness
- Uneven pigmentation
- Reduced water content (TEWL) and modification of lipids

prevalence of pathogens ?

Microbiome

The host's micro-environment plays a major role, any modification in skin physiology impacts on microbiota

Microbiome complexity is based on the uniqueness of individual micro-environment

# UV radiation plays a fundamental role in skin type evolution



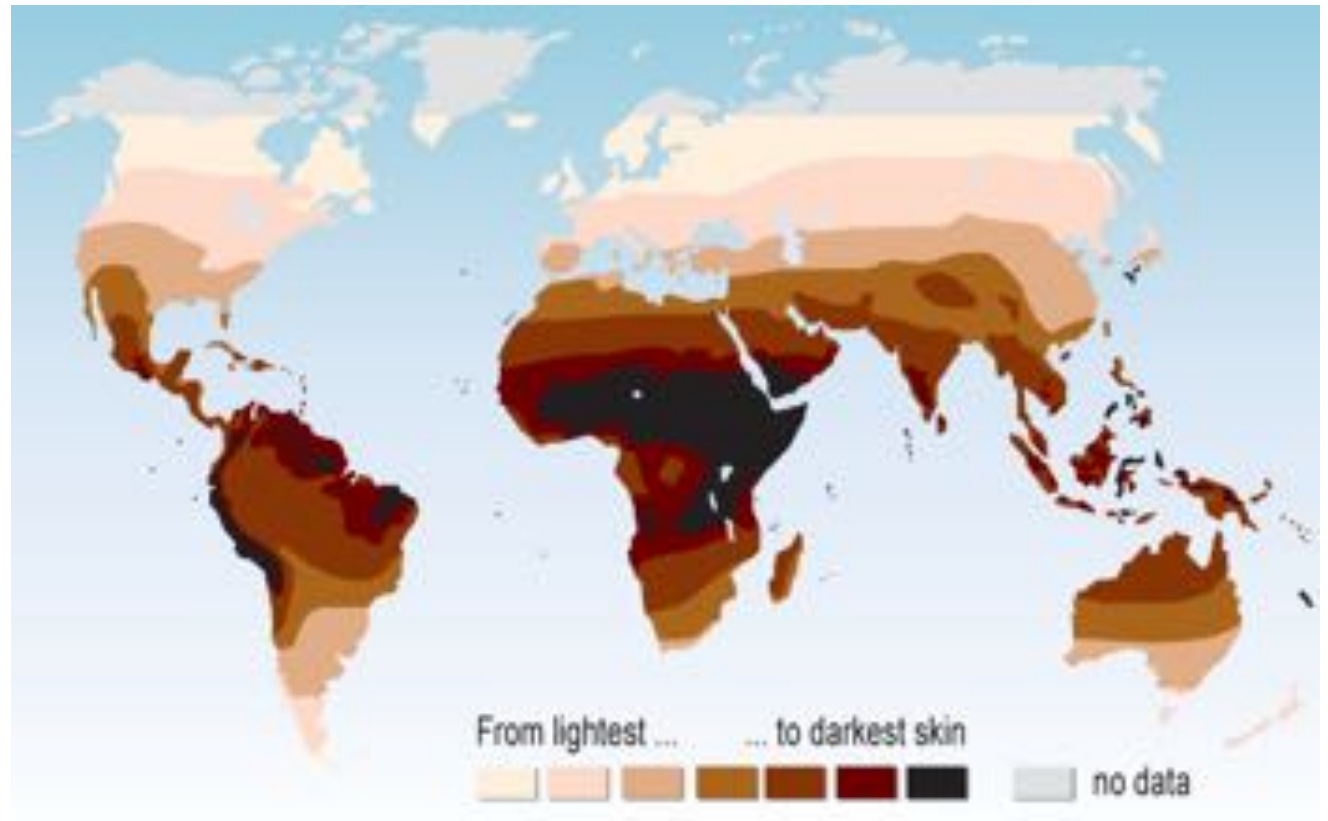
## SKIN DIVERSITY

### Phototypes

- Skin structure, SC composition;
- Barrier function;
- Sebum;
- Melanins;
- Circulating hormones;
- Sweat secretion;

## SKIN UNIQUENESS

because of skin micro-environment influencing  
**microbiome functional ecology**



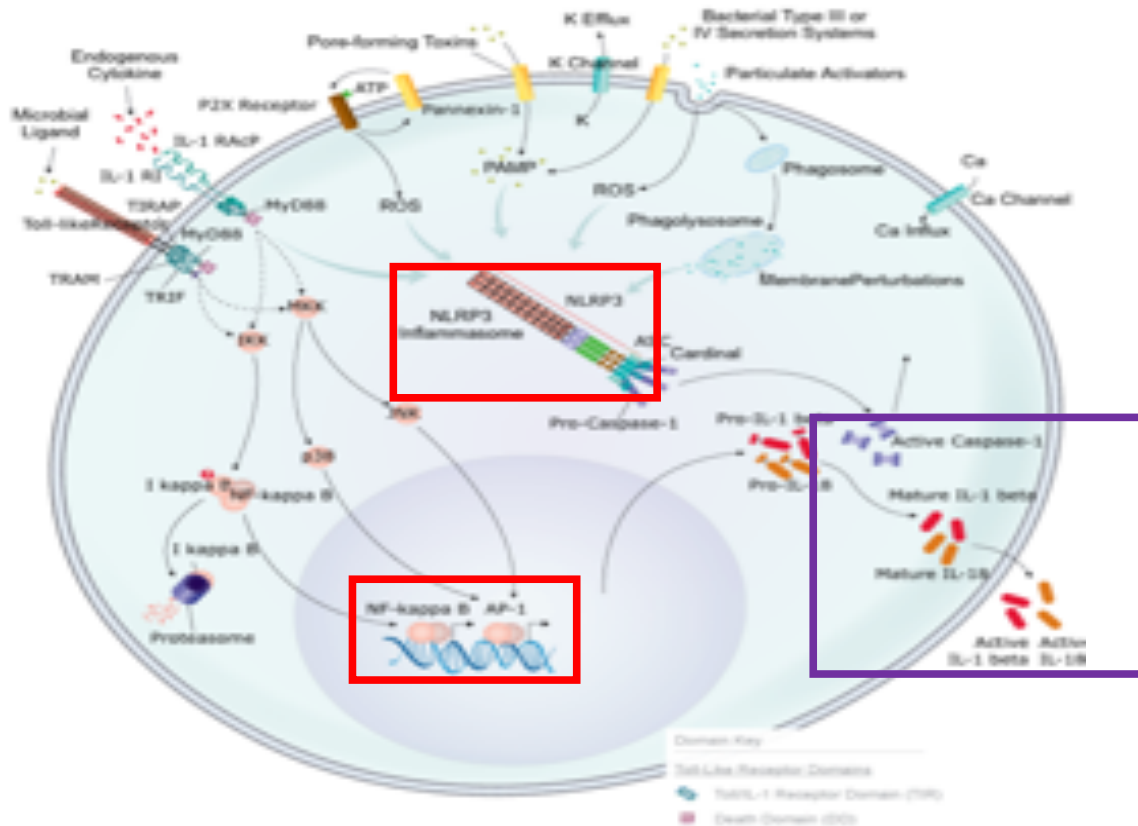
# INFLAMMASOME PATHWAYS: INFLAMMAGING

Relevant in Medicine in almost all therapeutic areas.

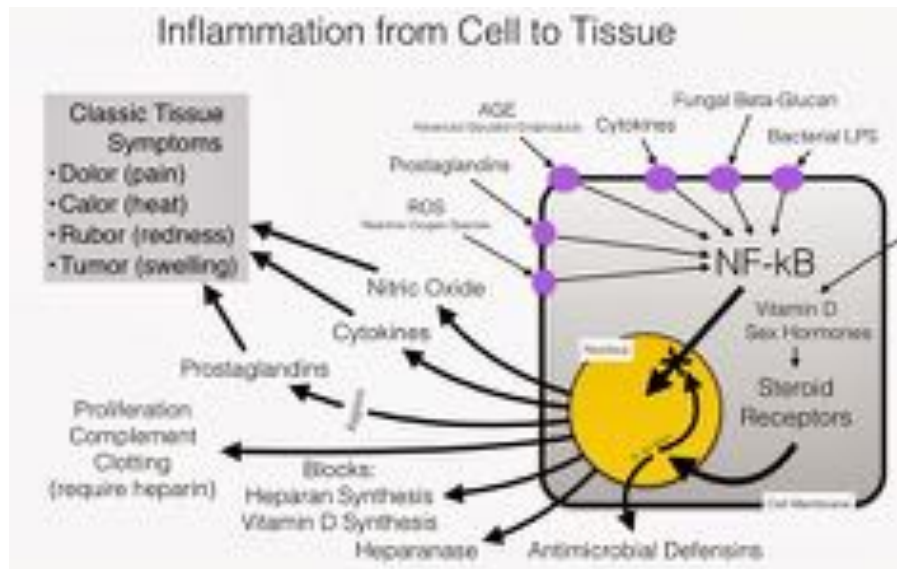
Age-associated low-grade inflammation (inflammaging) is recognised as being a driving force of many age-associated diseases linked to irreversible cellular and molecular damage that **is not clinically evident** because it slowly accumulates over decades.

Inflammaging is believed to be a consequence of a **re-modelling of the innate and acquired immune system**, resulting both in cumulative lifetime exposure to pro-inflammatory cytokines at older ages and production of reactive oxygen determining **modification to skin appearance and involvement in chronic diseases**.

(Baylis et al. Longevity & Healthspan 2013 2:8).



# INFLAMMASOME MASTER REGULATORS



**NLRP3:** it is the inflammasome major protein.

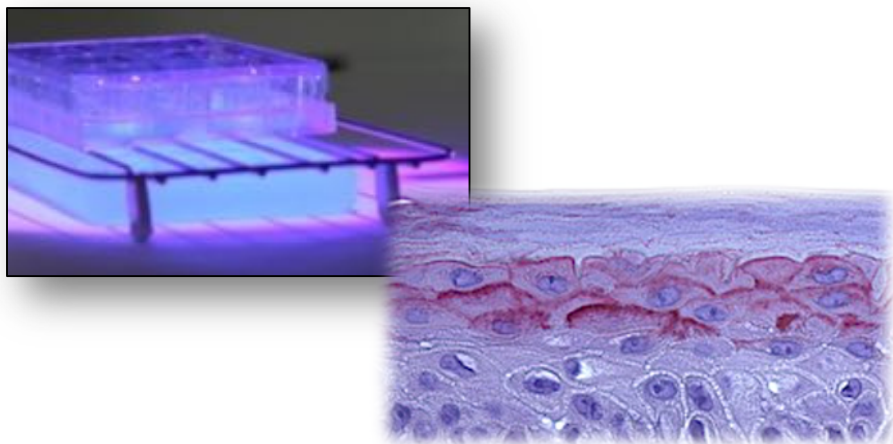
The discovery of the nod-like receptor protein (NLRP) inflammasomes in 2002 has led to the rapid identification of these unique cellular proteins as key targets for studies on innate inflammation pathways.

NLRP3 inflammasome activation is a kind of innate immune response.

However, if this activation is not properly regulated, excessive inflammation induced by over-activated NLRP3 inflammasome can be detrimental to the host.

**NF-kB** is a transcription factor and its **nuclear translocation** orchestrates cellular response to a stress induction and activate downstream cytokines expression.

# INFLAMMAGING: PRECLINICAL MODEL



Inflammasome biomarkers can be explored to figure out early biological events of **inflammaging**: it can be easily induced on 3D reconstructed skin models by relevant UV doses



## An Efficient Means to Mitigate Skin Inflammaging by Inhibition of the NLRP3 Inflammasome and NfKb Pathways: A Novel Epigenetic Mechanism

Hanane Chajra<sup>1</sup>, Sandrine Delaunoy<sup>1</sup>, David Garandeau<sup>1</sup>, Gaëlle Saint-Auret<sup>2</sup>, Marisa Meloni<sup>3</sup>, Eunsum Jung<sup>4</sup>, Mathilde Frechet<sup>1</sup>

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<sup>2</sup> Genel, Grenoble, France

<sup>3</sup> VitroScreen, Milano, Italy

<sup>4</sup> Biospectrum Life Science Institute, Yongin-City, Republic of Korea

*cosmetics*



Current Updates in Dermatological Problems

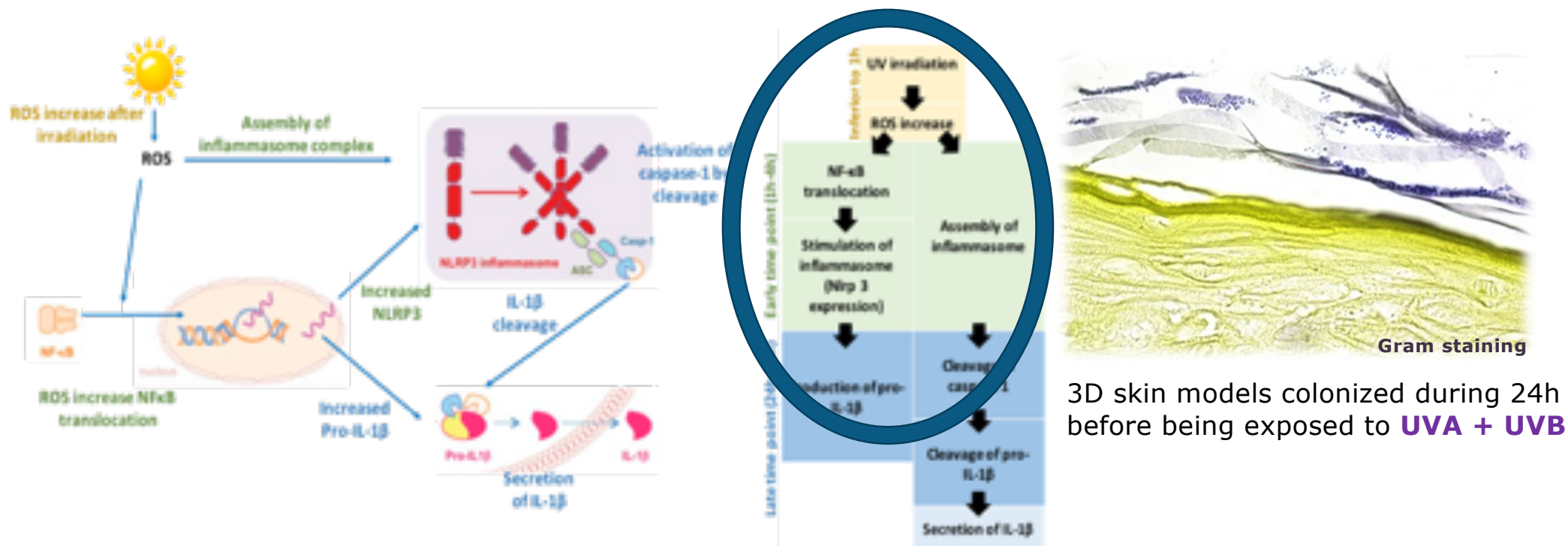
Synthetic Endocannabinoid as Anti-Inflammaging Cosmetic Active: an *In Vitro* Study on a Reconstructed Skin Model

L<sup>1</sup>\*, Meloni M<sup>2</sup>, Caviola E<sup>2</sup>, Galizia G<sup>3</sup> and Baratto G<sup>4</sup>

te: 09 December, 2018; Accepted Date: 28 December, 2018; Published Date: 07 January, 2019



# INFLAMMASOME follows a defined cascade...



## EXPERIMENTAL DESIGNS : 6H and 24H READOUT

- I. RHPE phototype II + *S.epidermidis*
- II. Ft- SKIN + *S.epidermidis* and *C.acnes*-pathogen
- III. RHPE phototype IV + *S.epidermidis* and *C.acnes*-commensal

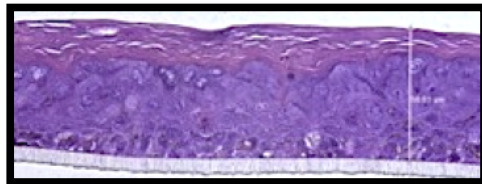
# I:*S. epidermidis* protects keratinocytes

RHPE II  
2 MED +6h

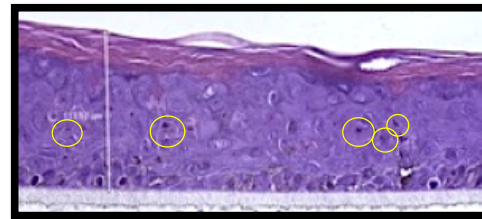
***S. epidermidis*** has shown a protective role in reducing inflammasome compared to germ-free RHPE

Skin microbiome's major component protects from early event of photoageing and inflammaging and it is able to regulate pigmentation.

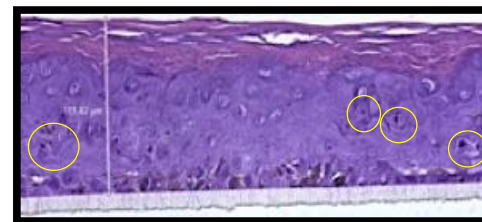
**H&E: Sun Burn Cells**



NEGATIVE CONTROL

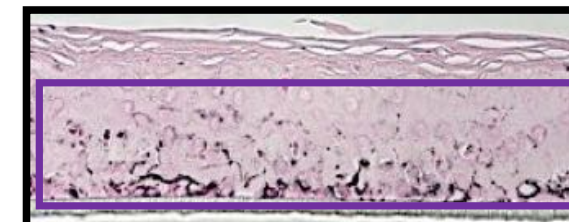
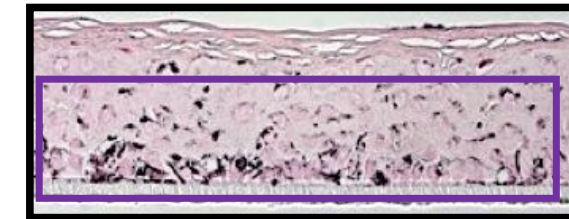
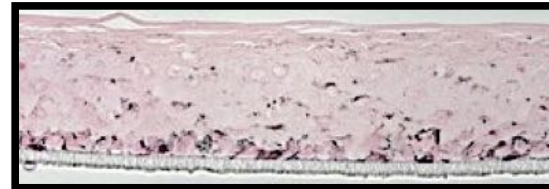


IRRADIATED 2MED

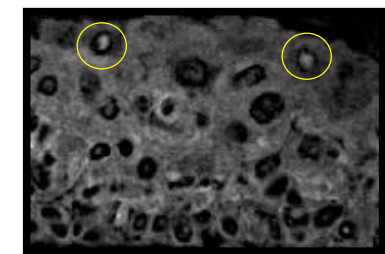
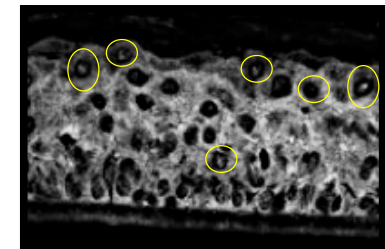
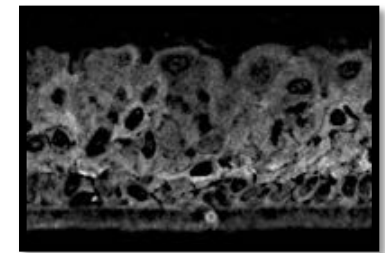


*S. epidermidis* + 2MED

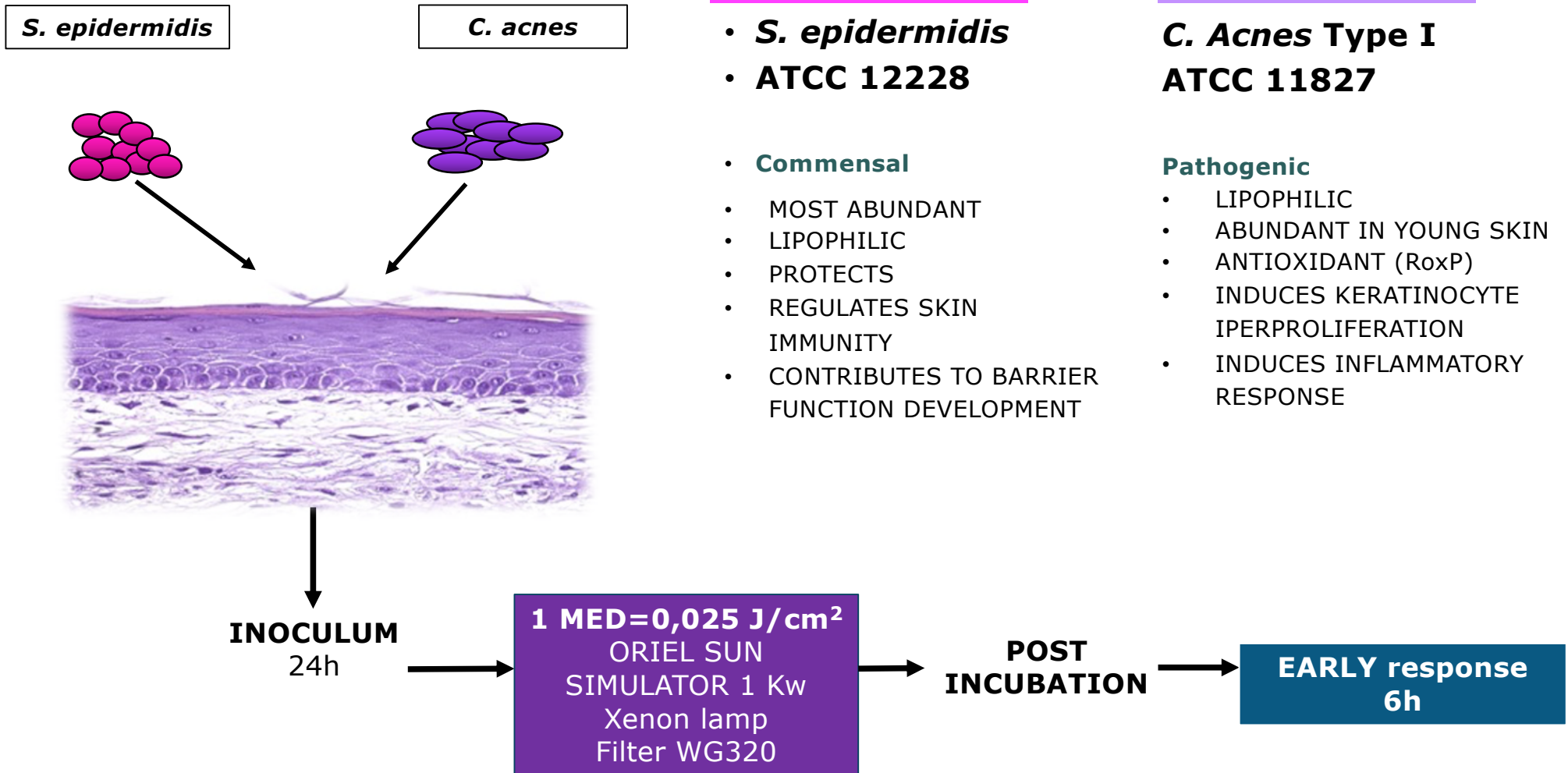
**Fontana Masson IHC**

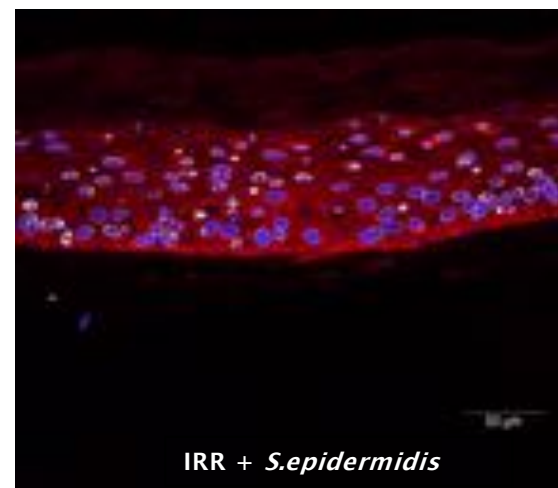
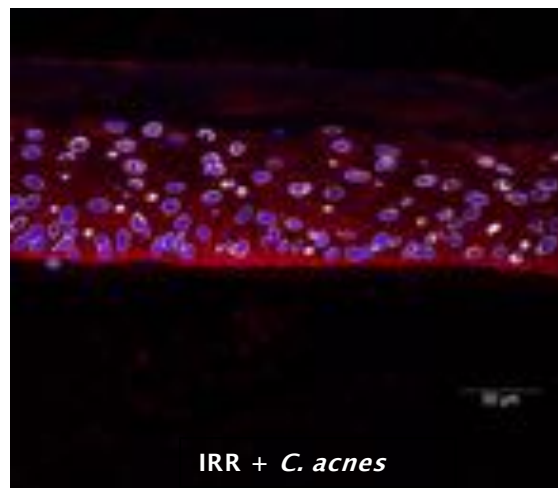
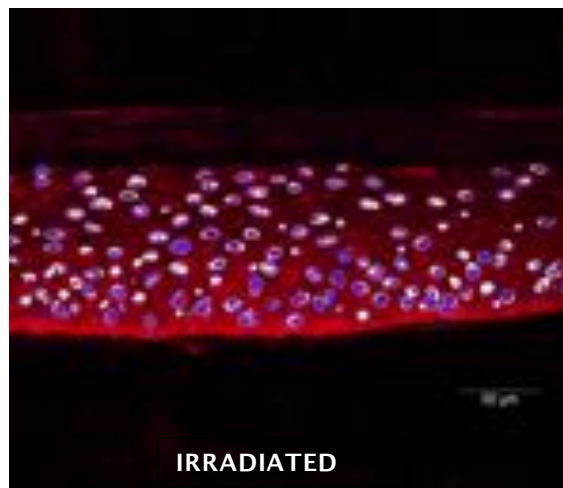


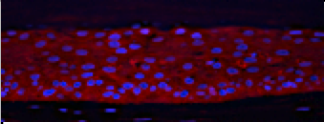
**NfkB Nuclear translocation**

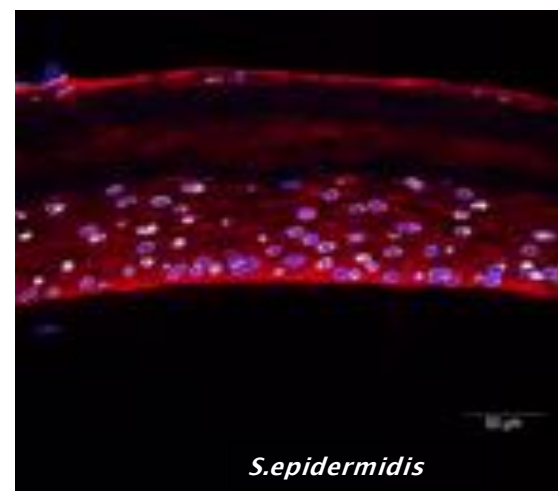
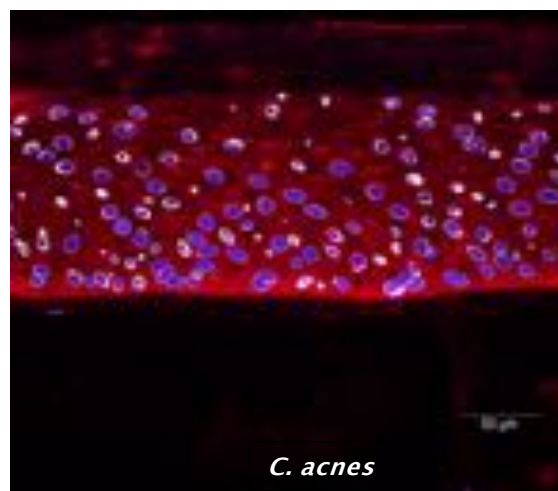


## II: *S. epidermidis* and *C. acnes* strain towards inflammasome activation



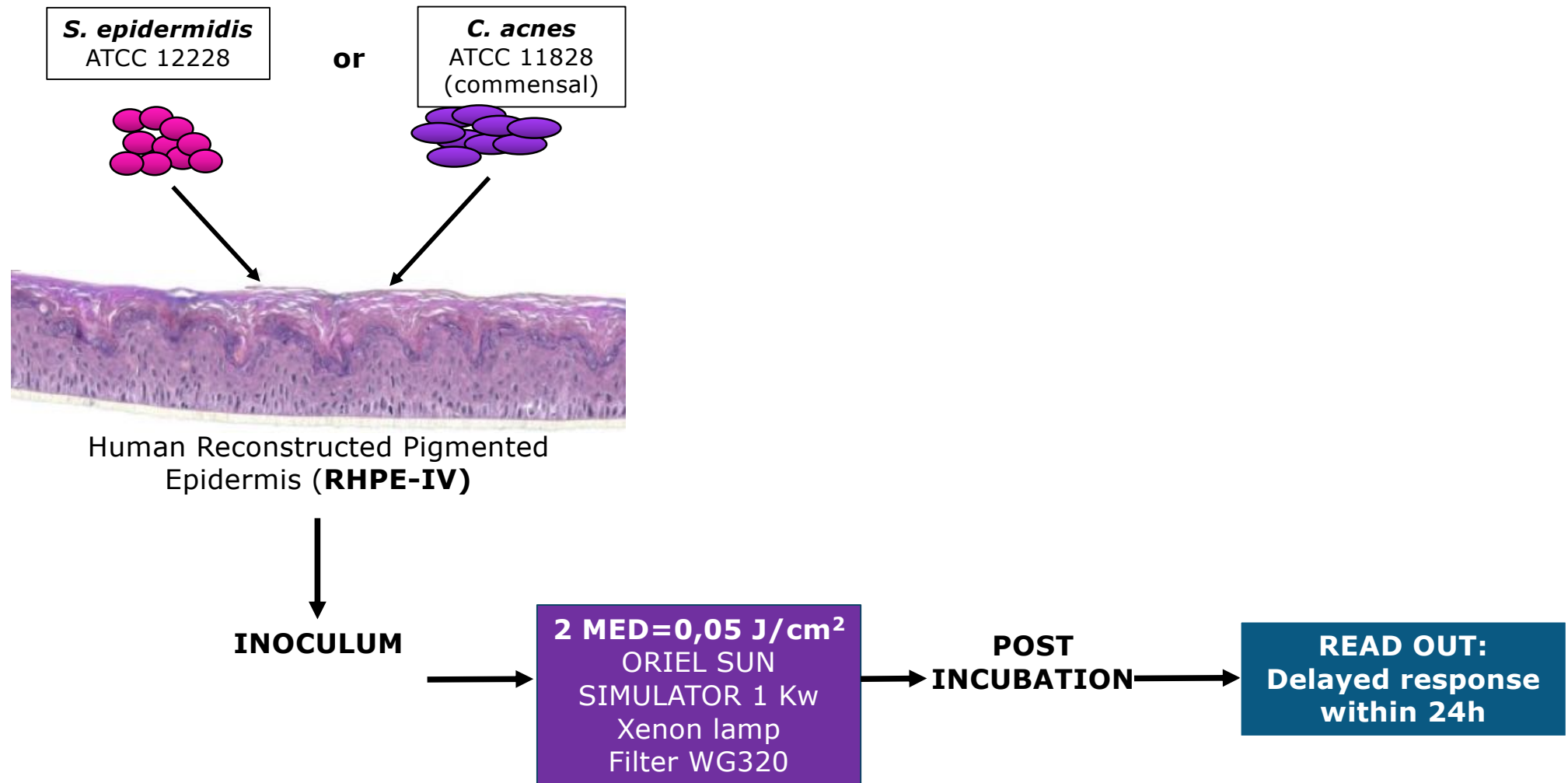


	NFKB nuclear translocation count 6 H
<b>IRRADIATED 2MED</b>	<b>256,5</b>
<b>C.acnes virulent</b>	<b>169</b>
<b>C.acnes +2MED</b>	<b>146</b>
<b>S.epidermidis</b>	<b>85</b>
<b>S.epidermidis +2MED</b>	<b>155,5</b>



Both strains have shown a protective efficacy  
against inflammasome activation  
Their impact on skin innate response is different,  
*C.acnes* has triggered an inflammatory reaction

### III: *S. epidermidis* and *C. acnes*-commensal



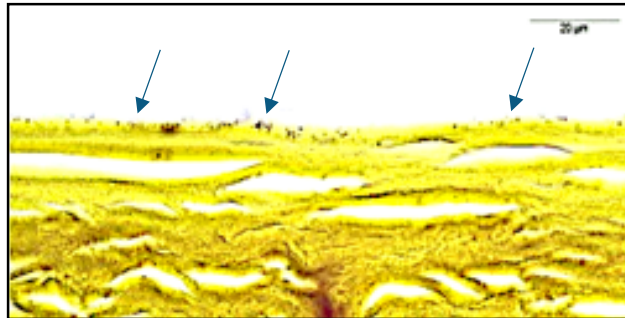
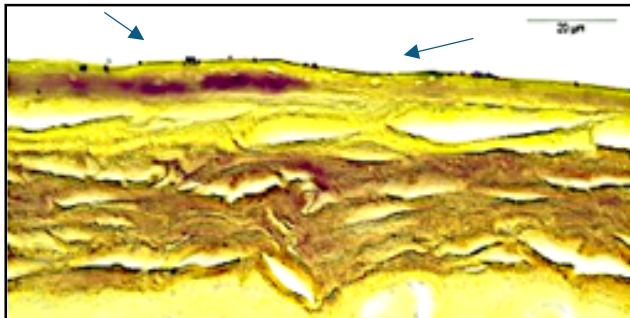
# III: GRAM STAINING

**100X magnification:  
2% of the entire tissue  
diameter (0,78 cm)  
is visible in each picture**  
**GRAM**

**RHPE IV COLONIZED**

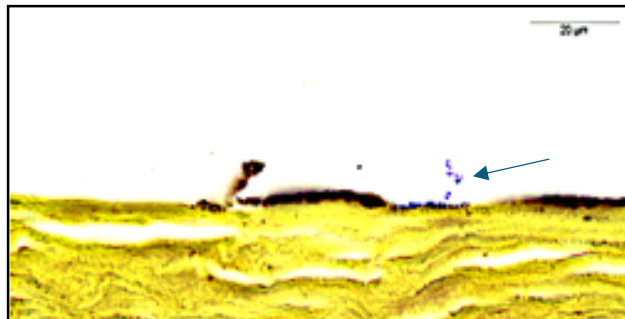
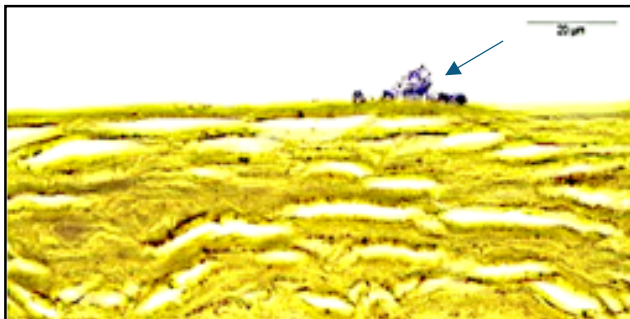
**RHPE IV COLONIZED+ 2 MED**

*S. epidermidis*



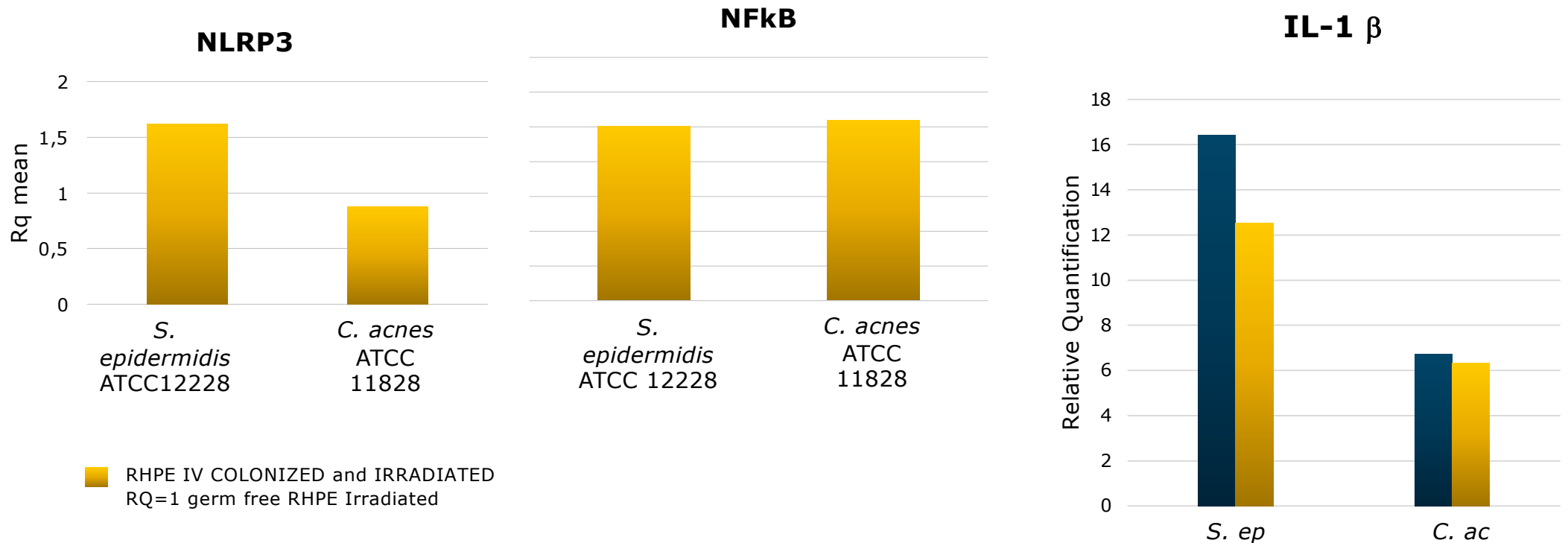
- *S. epidermidis* was evenly distributed on tissue surface;
- 2 MED decreases the number of bacterial cells confirmed by viable count but has not modified its distribution.
- *S.epidermidis* seems sensitive to UV

*C. acnes*



- Given its slow duplication time, *C. acnes* formed many bacterial clusters on SC.
- Bacterial cells are not decreased

# III: Inflammasome activation on RHPE IV : 24h

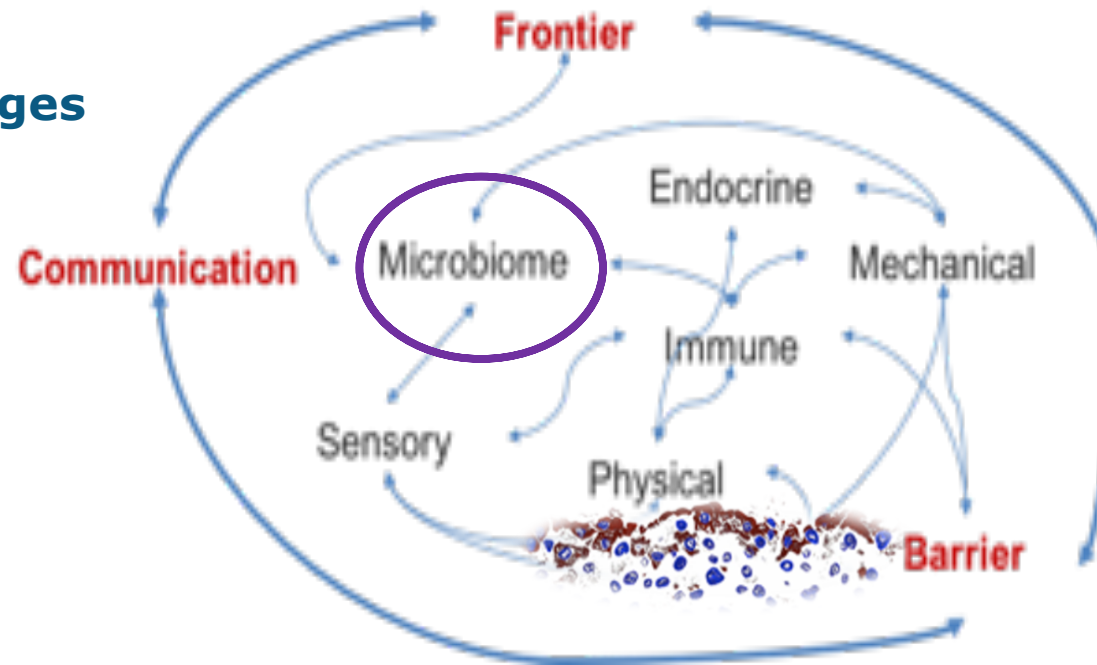


- ***S. epidermidis*** has activated NLRP3 expression (in absence of immuno-mediated response) suggesting a role of this bacterium towards innate immune-system via NLRP3 complex upstream cytokines activation ( **IL-1 beta**) and in boosting innate immunity.
- ***C. acnes*** did not activate NLRP3 expression compared to IRRADIATED control.
- **NFkB gene** is no more visible within 24h after 2 MED the biological effect
- **TLR2** were not activated (data not shown)

# Skin and associated microbiome are a complex community within an Evolutionary Organ: A Complex Barrier and Active Interface



Life's ages



In absence of pathologies ...does skin microbiome have a role in skin ageing ? **YES**  
As far as *S.epidermidis* is concerned it influences the very early stage  
of skin ageing process activating adaptive mechanisms to  
oxidative stress and inflammation

Role of *C.acnes* to be deeper investigated

# 3D COLONIZED HUMAN SKIN MODELS

Easy to handle in optimized experimental conditions

Short and Long term Colonization

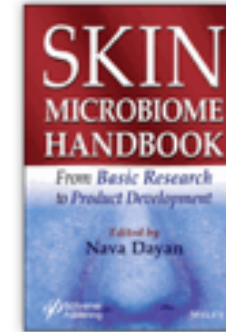
Single or multiple bacterium colonization (competition model)

Neutral/Naïve responses

SKIN DIVERSITY  
pathologies, young, old, ethnic..

Ethical, sustainable enabling routine testing capabilities

Biologically relevant



[Skin Microbiome Handbook: From Basic Research to Product Development](#)

Scientific knowledge of skin biology has been build up during decades in presence microbiota community but ignoring its contribution : experimental designs on colonized skin models can provide robust data to understand the microbiota **functional ecology mimicking host micro-environment.**

A scanning electron micrograph (SEM) showing a detailed view of skin cells. The image features several large, rounded, and somewhat irregular cells with a textured surface. Some cells are more prominent than others, showing their three-dimensional structure. The background is filled with a dense network of smaller, more fibrous structures, likely representing the extracellular matrix or other skin components. The overall tone is grayscale, typical of SEM images.

**To measure what is measurable and  
make measurable what is not so**

Galileo Galilei 1564 - 1641

The scientific community involved in skin microbiome research is contributing to write a new chapter in dermatology and cosmetology.

To do that we cannot apply the same tools used than before «*microbiome discover*» : host biology and bacteria functional ecology must be taken intoaccount.

Take home message:

- **Host response**
- **Role of single bacterium**



Thank you !  
Grazie !